T H E S I S

FACTORS CAUSING LOW GERMINATION IN SORGHUM SEED

Submitted by

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THIS THESIS HAS BEEN READ

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COLORADO AGRICULTURAL COLLEGE
AND EXPERIMENT STATION
FORT COLLINS, COLO.

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FACTORS CAUSING LOW GERMINATION IN SORGHUM SEED

Bruce J. Thornton

INTRODUCTION

The germination of sorghum seed on the average is very low, not only in the field but even under laboratory and greenhouse conditions. Though this germination may be even as low as 40 percent little study has been made of the cause of the decrease or of remedial methods that might be used to prevent it.

It will readily be recognized that a number of agencies may lower the germination of sorghum seed but a brief examination of the subject soon indicates that certain of these are outstanding. Mechanical injury, the accompanying excess of respiration in storage, and fungus invasion when the seeds are planted, play an important part in decreasing the germination. When it is realized that approximately 400,000 acres are annually planted to sorghums in Colorado and that to plant this acerage over 6,000,000 pounds of seed are used, a loss of 60 percent of this seed is of considerable

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economic importance. Of even greater importance may be considered the loss resulting from poor stands which are often so low as to necessitate replanting with the consequent increased cost and liablity of lessend yields due to the shortened growing season.

It is the purpose of the following discussion to present data explaining the influence of the factors causing this loss from low germination and to suggest means of decreasing it.

LOW GERMINATION OF SORGHUM SEED.

Before considering the causes of low germination in sorghum seed, the records of the State Seed Laboratory were consulted for germination tests of sorghum samples received during the years of 1921 to 1925 inclusive. Tests were also made of individual samples of seed to determine the extent of seed injury. The laboratory furnished the seed material used which was taken from samples sent in from the fields for the years 1924 and 1925 respectively. This material was very representative of the sorghums grown in Colorado and consisted of both saccharine and grain types. The records previous to 1924 were utilized to show the average germination of sorghums over a period of years while more complete data were worked up from the 1924 and 1925 records because these records represented material actually at hand and available for experimental use.

In the following table is shown the characteristic germination of saccharine and grain sorghums as determined from several years' records of the State Seed Laboratory.

Table 1. Laboratory germination records of Sorghum seed for the years 1921 to 1925.

Year Grown	Number Samples	Percent germination		
		Maximum	Minimum	Average
1921	70	98.5	15.0	73.2
1922	132	99.0	9.0	75.5
1923	124	98.5	0	64.7
1924	104	99.0	5	73.4
1925	139	98.3	6.9	71.1
Average		98.3	6.9	71.1

It is evident from this table that sorghum seeds, in general, germinate very poorly even under the ideal conditions furnished in the laboratory. That this does not hold for all samples is apparent from the column of maximum germination showing that some samples germinated as high as could be expected of any seed.

Field germination as compared to the laboratory germination is shown in Table 2.

Table 2. Laboratory and field germination of 104 samples of Sorghum seed (1924 seed).

Germinated	No. samples	Percent germination		
		Maximum	Minimum	Average
Laboratory	104	99	5	73.4
Field	104	75	0	38.0

In the above table it is seen that under laboratory conditions the maximum germination was 99 percent, the minimum 5 percent, with an average germination of 73.4 percent. Under field conditions the maximum germination was 75 percent with a minimum of 0 percent and an average germination of 38 percent.

This not only again brings out the low average germination of the sorghums but, in addition, shows the striking deterioration of the seeds under field conditions, the samples that averaged 73.4 percent in the laboratory doing but little better than half as well in the field.

In studying the germination of the various sorghum samples comparison of the relative germination of the grain and saccharine sorghums was made. Such a comparison is shown in Table 3. which is taken from the laboratory and field records of thirty samples of grain sorghums and sixty-six samples of saccharine sorghums.

Table 3. Germination of saccharine and grain sorghums in the laboratory and in the field.

	No. of samples taken	Percent Germination					
		Laboratory			Field		
		Maximum	Minimum	Average	Maximum	Minimum	Average
Grain	30	99	17.5	83.70	75	0	35.50
Saccharine	66	95	5	68.60	73	6.0	40.70

That the grain sorghums have a higher percent germination than the saccharine sorghums under laboratory conditions is indicated in this Table; the grain samples averaging 83.7 percent germination and the saccharine sorghums averaging 68.6 percent. However, this same table shows that in the field the reverse is true, the grain sorghums averaging 35.5 percent, which is a decrease of 48.2 percent from the laboratory count, while the saccharine sorghums germinated 40.7 percent, a decrease of only 27.9 percent from the laboratory germination. In other words, field conditions decreased the germination of the grain sorghums 42 percent more than they did the germination of the saccharine sorghums.

From this data it appears that the germinating powers of the saccharine sorghums are normally lower than those of the grain sorghums but that they are considerably less subject to the deleterious effects of field conditions. At present no explanation of this is offered other than the supposition that the persistent hull, which characterizes the saccharine sorghums, may afford some protection against soil factors unfavorable to seed germination.

FACTORS INFLUENCING THE GERMINATION OF SORGHUM SEEDS.

Work done in the past with seeds other than sorghum seeds has shown that germination is dependent upon several different favorable factors and likewise that there are many unfavorable factors which may operate to cause low germination. These unfavorable factors may work independently of each other but are undoubtedly more damaging as a result of their concerted action. Among the more important factors of this nature, affecting the mature seed, may be considered mechanical injury incurred in threshing and handling, rate and amount of respiration in storage, and the attacks of various organisms in the soil. In working with the sorghums the matter of seed maturity appeared to bear an important relationship to germination ability but time did not permit its study. The observations made would indicate immature seeds to be of especially low germination.

RELATION OF MECHANICAL INJURY TO GERMINATION

A study of individual samples of sorghum seed show that there is present in all of them, a large amount of cracked and broken seed. This condition coupled with low germination of these samples suggests such injury as a possible cause of low germination. Several workers have noted the poor germination of injured seed. Zapparoli (33) worked with a break

in corn, which is a type of defect occurring when kernels are hardening and claimed this injury to be the main cause of serious deterioration during winter storage.

Bailey (3) found that the moisture content of corn determines, to a large extent, its rate of respiration and that cracked and broken kernels respire much more vigorously than do sound kernels. Ringelmann (24) summarized data from different sources to show that machine threshing of seed grain has a tendency to so injure it mechanically as to reduce its germinating powers. The larger seeds were found to suffer greater injury than the smaller ones.

The relation of seed injury to germination as indicated in the above citations no doubt plays a part in lowering the germination of sorghum seed. This injury may be of such proportions as to chip off large pieces of the seed coat, break away a large part of the endosperm, or destroy all or part of the embryo. Examples of such breakage are shown in Plate I. The types of breakage are not recognized by the seed analyst other than to throw out and class as "inert matter" all seeds less than half. If more than half of the original seed is present it is considered as a whole seed regardless of the nature or type of the break.

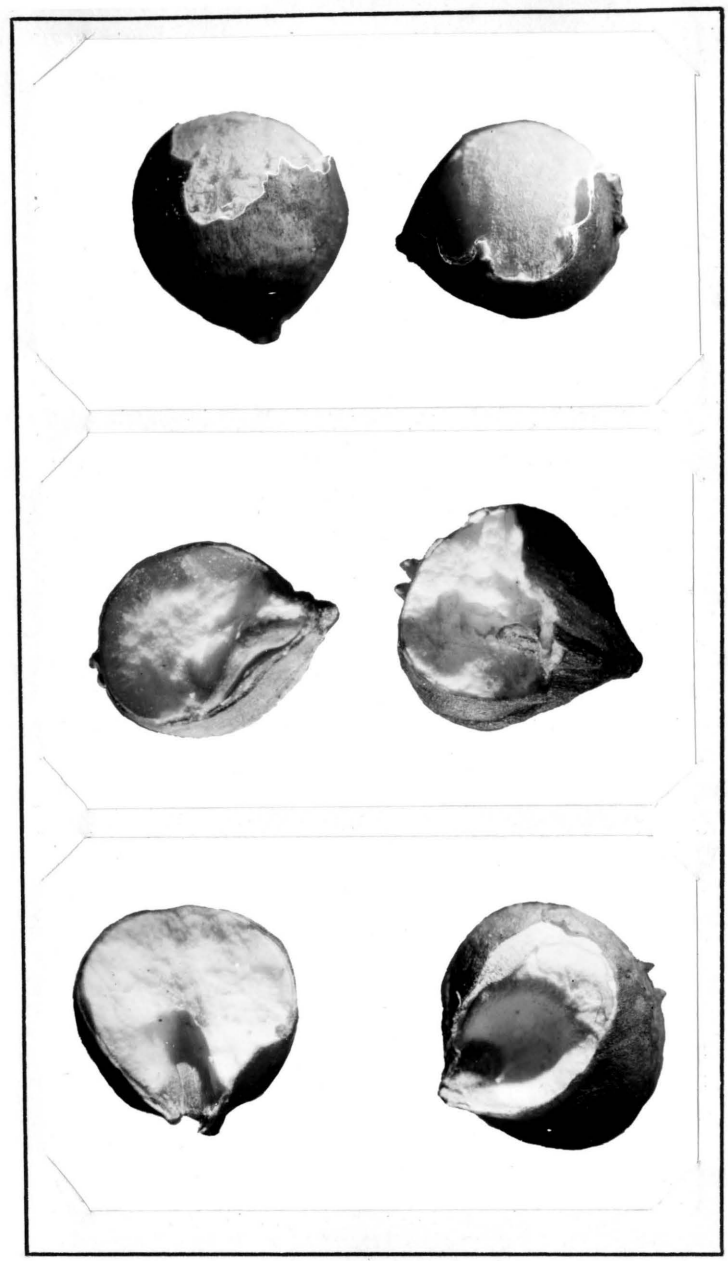

Plate I.
Types of seed injury:

Upper - Portion of seed coat chipped off.
Middle - Large piece of endosperm broken away; embryo present, often exposed.
Lower - Large piece of endosperm broken away; embryo destroyed.

STRUCTURE OF SEED COAT.

It is interesting in this connection to note the general structure of the seed coats of representative sorghums as illustrated by camera lucida drawings shown in Plates II and III. Plate II is a cross section of the seed coat of a grain sorghum and Plate III shows the cross section of the seed coat of a saccharine sorghum.

As shown by these drawings, the seed coat of the sorghums when considered, in the light of the caryopsis, as including all the structure outside the endosperm, consists of the pericarp (P) and the nucellar layer (N) . The pericarp is made up of the cuticle (c), the epidermis (e), the hypodermis (h), a mesocarp layer (m) of large thin walled parenchyma cells filled with small starch grains, a layer of cross cells (cr) and a layer of tube cells (t). The nucellar tissue consists of a single layer of large hyaline cells with comparatively thin sides walls and heavier inner walls which may be considerably thickened and pigment containing as shown in Plate V. This layer was not present in all the varieties examined. The endosperm (E) shows the characteristic aleurone layer (a) beneath which are the large thin-walled starch-containing parenchyma cells (st).

In the work with the seed coats it was noticed that the breaks always occurred thru the mesocarp layer of thin-walled

cells or thru the thin walls of the nucellar layer. Seeds in which the nucellar layer was lacking seemed to be especially free from breakage. Observations made also indicated that there was a lower percentage of breakage in seeds in which the cells of the epidermis and hypodermis were of a more compact structure as in Plate III than when these cells were larger and less compressed as shown in Plate II.

The time available did not permit sufficient work being done upon the structure of the seed coat to warrant the drawing of any definite conclusions as to the relation of this structure to the prevalence of seed injury. However, such observations as were made appear to indicate that such a relation does exist and seem to warrant more extensive work on the subject.

DATA ON RELATION OF SEED INJURY TO GERMINATION.

In order to determine whether there is any correlation between this peculiar mechanical injury and low germination ten samples were selected at random and the whole, chipped, and broken seed separated. Germination tests were then made of these seed, the record of which is shown in Table 4.

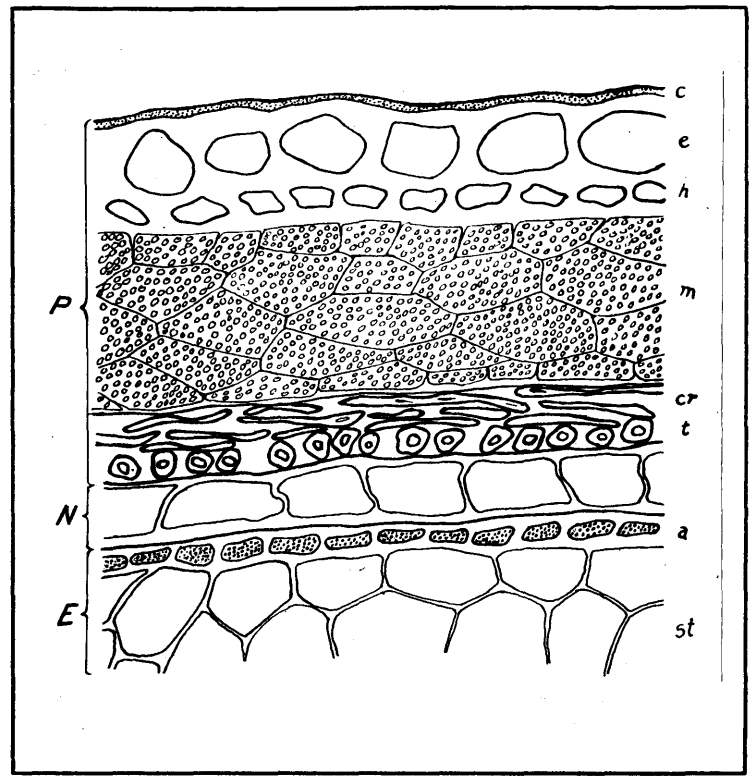

Plate II.

Cross section of seed coat of grain sorghum.

P, pericarp consisting of cuticle c, epidermis e, hypodermis h, mesocarp m, cross cells cr, and tube cells t.

N, nucellar layer

E, endosperm showing aleurone layer a, and starch cells st.

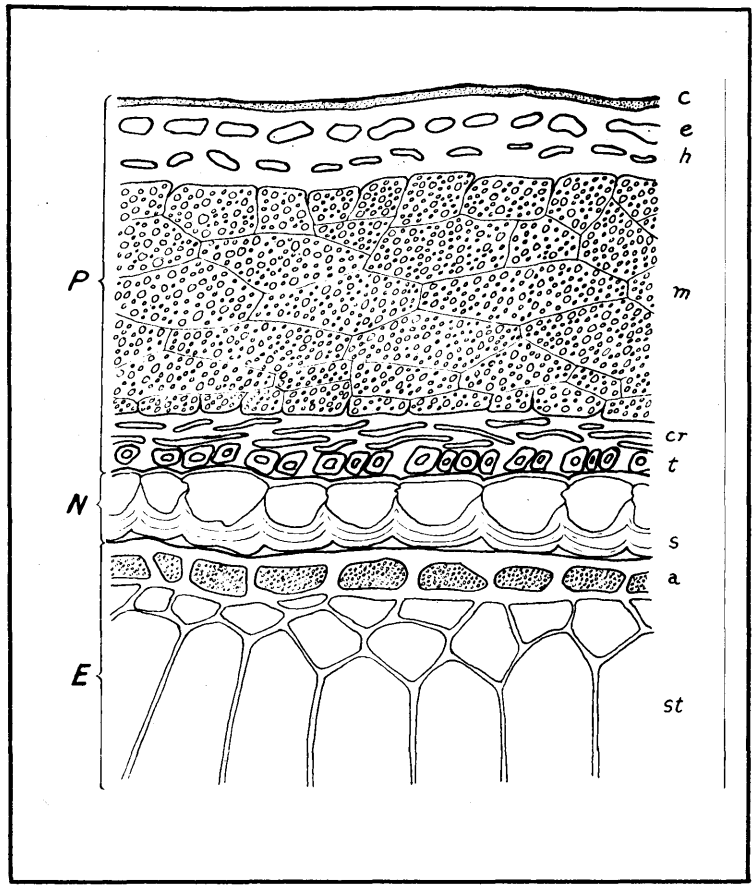

Plate III.

Cross section of seed coat of saccharine sorghum.

P. pericarp consisting of cuticle c, epidermis e, hypodermis h, mesocarp m, cross cells cr, and tube cells t.

N, nucellar layer showing swollen inner walls.

E, endosperm showing aleurone layer a, and starch cells st.

Table 4. Germination of whole and Injured seed.

Sample Number	Percent Germination		
	Whole	Chipped	Broken.
67	69	15	5
68	88	37	6
72	56	29	5
92	79	17	2
96	77	21	4
97	85	25	2
100	81	31	2
104	87	41	7
105	80	32	0
64	94	25	8
Average	79.6	27.2	3.9

It is obvious from the above table that the injured seed germinate poorly. Both broken and chipped seed are markedly lower in germination than whole seed. The average germination of broken seed being but 3.9 percent while chipped seed germinate but 27.2 percent as against a 79.6 average germination for whole seed, the germination being to a large extent proportional to the degree of injury.

Though in some samples there was some suggestion that injury over the embryo end of the seed was more deleterious to germination than injury elsewhere and this behavior would seem reasonable, experiment does not bear out the point.

In Table 5 are shown records of tests on six samples of sorghum, some of the seed having injury at proximal end and some at distal end.

Table 5. Germination with regard to location of injury.

Sample Number	Percent Germination		
	Whole seeds	Broken at proximal end	Broken at distal end.
1	62.5	0	3.5
2	77	35	26
3	83	16.5	15
4	81	3.0	9.5
5	100	12.5	13
6	87	4.5	12.
Average	81.7	11.9	13.1

It may be seen in the above table that on the average little difference in germination results from breakage of either proximal or distal ends of the seed.

Broken seed comprise the bulk of so-called impurity in samples of sorghum seed. Careful examination of sorghum samples shows that the purity test, as made in the Seed Laboratory, does not take account of all injured seed, only those seed less than half being recognized and separated out. Therefore, in Table 6 are given figures on the purity, germination and percent of whole, chipped and broken seed in eight representative samples of sorghum seed.

In Table 6. the evidence indicates that no consistent relation exists between purity and germination. In sample 140 with 77 percent purity but 31 percent of the seed germinated. In sample 38 with 88 percent purity, 91.5 percent germinated, while sample 115 only 65 percent germination is coupled with a 95 percent purity. At first thought this suggests that our

Table 6. Percent whole, chipped, and broken seeds as Compared to germination and purity.

Sample Number	Laboratory		Number of seeds	Whole seed		Chipped seed		Broken seed	
	Purity percent	Germination percent		Number of seeds	Percent of sample	Number of seeds	Percent of sample	Number of seed	Percent of sample
38	88	91.5	274	160	58	87	32	27	10
50	93	51	305	270	89	21	7	14	4
53	93	80	266	226	85	31	11.5	9	3.5
55	93	89	248	237	95.5	8	3.3	3	1.2
65	90	78	380	350	92.2	24	6.3	6	1.5
115	96	65	245	218	89	16	6.5	11	4.5
132	95	17.5	369	350	95	2	.5	17	4.5
140	77	31	192	161	83.9	16	8.3	15	7.8

definition of "purity" is too gross and includes only badly broken seed, as is the case, and that the injury incident to chipping and scratching of the seed accounts for the difference. The data presented in the above table, however, shows for instance, that sample 38 has 32 percent chipped seed but germinated 91.5 percent, while sample 132 with but .5 percent chipped seed and 4.5 percent broken seed germinated 17.5 percent. Many similar records might be brought to evidence this relation.

Although it is apparent from these data that there is a greatly reduced germination as the result of purely mechanical injury, the location of the injury seems to make but little difference. It is also evident that the matter of breakage is not always the controlling factor, but that there are other factors or combinations of factors which may exert a much greater influence.

RESPIRATION STUDIES.

Poor germination of sorghum seed may in part be considered the result of changes in respiration rate in the seed. This would seem to be especially true of scratched or broken seed.

Numerous references are extant on the respiration of seeds and other plant tissues. Sinnot (26) states that

evolution of CO_2 even in minute quantities is regarded as proof that the organism is respiring and therefore alive and Palladin (22) observes that the respiration rate decreases on account of the diminishing of the supply of carbohydrates. Duvel (8) carried on extensive studies on the viability and germination of seeds and in speaking of the way in which the presence of increased quantities of water causes their premature death, says: "In a measure the answer to this question is respiration. Seeds, as we commonly know them, absorb O and give of CO_2, that is, respire. During these respiratory activities the energy stored within the seed is readily evolved, the vital processes are destroyed and life becomes extinct." Hausman and Ivanicot (16) after having investigated the relations between respiration and germinability in seeds stated that a certain relation must be admitted to exist between percent germination of cereal seeds and the CO_2 elaborated during respiration but that it was impossible to determine, at that time, the relation exactly. Harrington (12) found that the removal of the outer seed coat or both seed coats in the case of apple seeds greatly increased the respiratory intensity. Shull (28) in testing the semipermeability of seed coats found that there was no measurable diffusion of oxygen thru the dry coat but that the slightest injury to the coat permitted a

rapid passage of oxygen. Of interest in this connection are the results of this same worker (29) in ascertaining the role of oxygen in the germination of seeds. Here he determined that much less energy was released in anaerobic than in aerobic respiration and that access to oxygen may change the former to the latter with consequent increase in energy release. Lack of oxygen apparently acts as a limiting factor either by limiting the process of respiration or energy release, by limiting enzyme formation of the action of oxygen carriers, or in other still less definite ways. Atwood (2) in observing the germination of Avena fatua, stated that with increased germination rates there was increased ability on the part of the seed to take up oxygen and that wounding actually increased the oxygen intake, while Tashiro (30) was able to measure the evolution of CO_2 from very small scratches in seed coat. The studies of Swanson (27) on the relation of the seed coat of Feterita to the rate of water absorption and germination evidenced the fact that water was absorbed more readily by Feterita and other sorghums having soft seed coats than by those varieties with hard, glossy coats and as a result they tend to rot and germinate poorly in damp cold soil altho capable of giving good germination in comparatively dry seed beds.

METHODS USED IN RESPIRATION TESTS.

In determining the possible effect of rapid or prolonged respiration as affecting the germination of sorghum seed, tests were made of the evolution of CO_2 from the seed as an index of viability. Several methods and types of apparatus were considered.

A modification of Truog's method as suggested by Gurjur (11) was used in a simplified form but was abandoned because of its complexity and because of the limited number of samples that could be handled in a given time. The indicator method as used by Parker (23) in his work with the frog's nerve suggested possibilities especially if work was to be done with single seeds. Since no workof this type was done and because of the limitations of the apparatus otherwise its use never got beyond the experimental stage. A type of apparatus in which the germinating seeds were suspended in a glass vial over a caustic soda solution all within a 50 cc. Erlenmeyer flask also proved unsatisfactory because of lack of uniform gas and moisture distribution among the seeds even when the bottom of the vial was pierced to provide for this.

The type of respirometer finally adopted may be considered as somewhat of a modification of the one described by Harrington and Crocker (14) in their work with seeds. Of necessity it is much cheaper in construction and not being equipped with a manometer it can only be used for measuring the

carbon dioxide evolved. The simplicity and reasonable cost, however, permit the construction of a large number of individual respirometers and thus enables the investigator to work with a great many samples where he is concerned with the measurement of respiration as evidenced by the CO_2 produced.

DESCRIPTION OF APPARATUS.

The apparatus (Fig. 1) consists of a glass shell (A) 9 cm. deep by 3½ cm. in diameter, closed by a number 7 rubber stopper (B), from which is suspended by means of a copper wire (C) a perforated paper cup or seed container (D) which is 1½ cm. deep and of such a diameter as will fit nicely within the shell. The wire and cup are dipped in melted paraffin to prevent any electrolytic effect upon the part of the copper and also to render the cup impervious to moisture.

CO_2 free barium hydroxide is introduced into the shell by means of the apparatus (Fig. 2) which consists of (1) a set of 3 wash bottles (A) containing NaOH solution; (2) a storage vessel (B) containing the $Ba(OH)_2$ solution, tightly stoppered and provided with inlet (C) from the wash bottles and an outlet in the form of a siphon (D) closed by stopcock (E); (3) a calibrated glass tube (F) supported by a ring stand (G) and also closed by a stopcock (H)

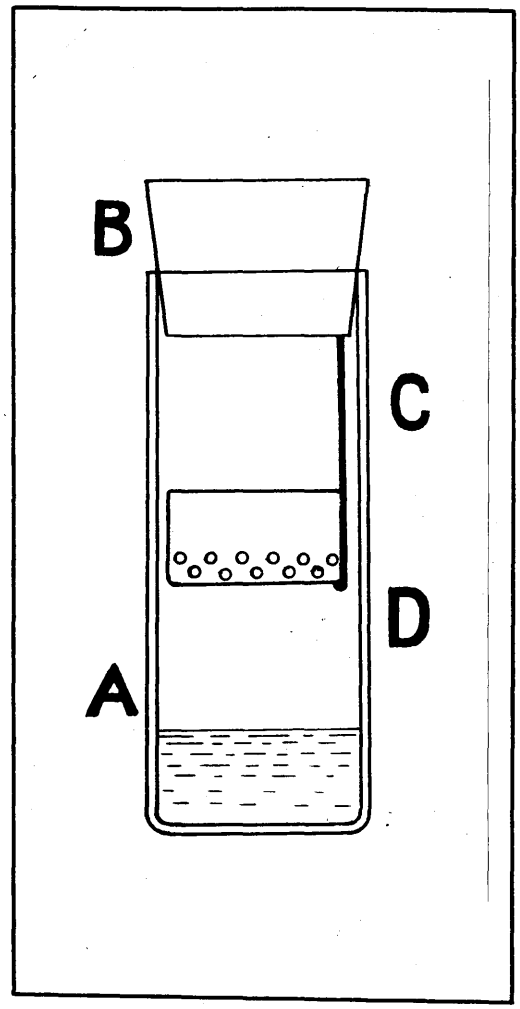

Fig. 1. Diagram of respirometer used in germination tests

A. glass shell
B. rubber stopper
C. copper wire
D. perforated paper seed container.

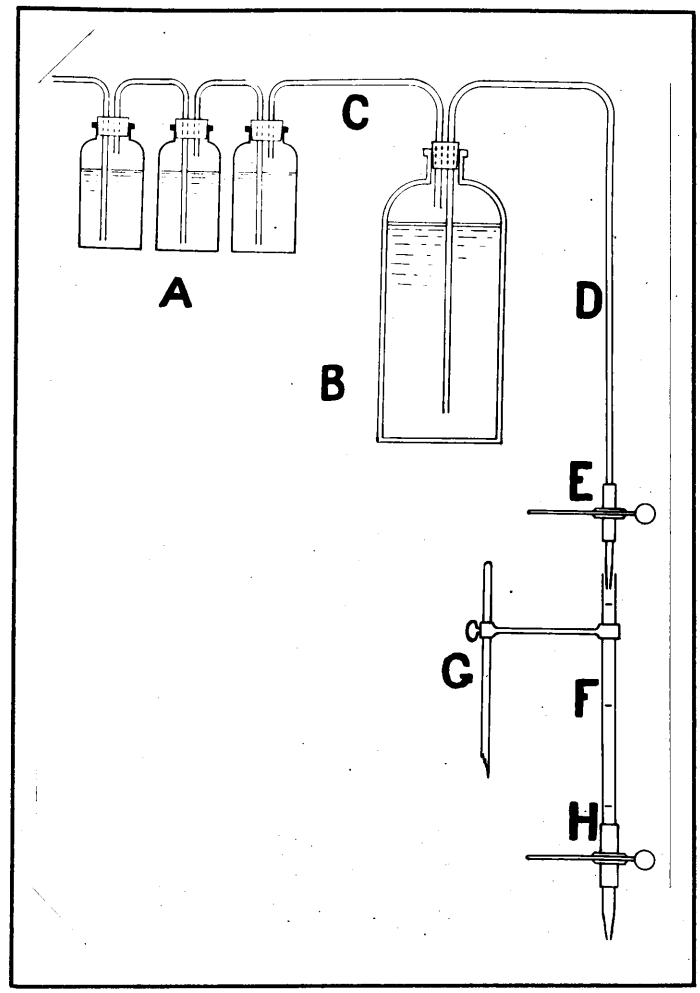

Fig. 2. Drawing of apparatus for introducing CO_2 free Barium hydroxide into respirometer:

A. wash bottles
B. storage vessel containing Barium hydroxide
C. CO_2 free air inlet to storage vessel from wash bottles
D. siphon outlet from storage vessel
E. stopcock
F. calibrated glass tube
G. ring stand
H. stopcock.

OPERATION OF APPARATUS

Five cc. of the saturated $Ba(OH)_2$ solution is introduced into the shell (Fig. 1,A) from the storage vessel (Fig. 2,B) by means of the siphon (Fig. 2,C) and measuring tube (Fig. 2,E) which is calibrated at 5 cc. intervals. Entrance of CO_2 into the storage vessel is prevented by causing the inflowing air to be drawn thru the set of wash bottles (Fig. 2, A) containing sodium hydroxide solution. The shell is quickly stoppered with an ordinary No. 7 stopper, several shells being filled at a time by this method.

A measured weight of imbibed and sterilized seed is then placed in the seed container and at once transferred to the shell containing the barium hydroxide solution by simply substituting the stopper bearing the seed cup for the one closing the shell. In this manner any number of seed samples are placed in their respective respirometers, all put in a suitable holder and placed under controlled germination conditions. At the end of any respiration period the germinating seeds may be transferred to a new shell with its fresh supply of barium hydroxide solution while the old shell is quickly stoppered after which it may be set aside and the amount of CO_2 absorbed from the respiring seeds calculated at the worker's convenience.

By this method the respiration tests of a given sample of seed may be continued as long and the measurements of CO_2 evolved taken as often as deemed advisable in order to observe

any changes in the rate of respiration. Care must be taken in exchanging shells to prevent the absorption of CO_2 from the air during the process and likewise the individual periods should not be of such lengths as to inhibit the action of the seeds thru lack of oxygen in the shell. That there is ordinarily little danger of this is indicated by Jost (18) when he states that respiration is not effected by reduction of oxygen pressure until the amount of oxygen present in the air is less than 2 percent. As pointed out by Gurjar (11) , 1 cc. of N/4 $Ba(OH)_2$ is equivalent to 6 mg. of CO_2. The saturated solution of $Ba(OH)_2$ used in this work has, at the temperature prevailing, a normalcy of approximately 1/2.5 and the 5 cc. used per shell is equivalent to 47.5 mg. of CO_2 . This provides a safe excess of the caustic solution for measuring the CO_2 evolved by a half gram of seeds for periods up to six days without the necessity of changing the solution.

The amount of CO_2 absorbed by the $Ba(OH)_2$ was measured by the titration method presented by Scott (25) and later modified by Harter and Weimer (15) who, in order to obtain more definite end points, used Thymol Blue (thymo sulphonthalein) and brome phenol blue (tetra bromo phenol sulphonthalein) as indicators in place of the phenolthalein

and methyl orange suggested by Scott.

In this method the excess $Ba(OH)_2$ is carefully neutralized by the addition of N/4 HCl from a burette using the thymol blue as the indicator. The precipitate of $BaCO_3$ is then dissolved by adding a measured excess of N/10 HCl from a burette. The resulting solution is then titrated against N/10 NaOH solution, using the brom phenol blue as the indicator. The amount of N/10 NaOH necessary to bring the solution to the neutral point gives the equivalent of the excess acid added in dissolving the precipitate and this amount subtracted from the total amount of acid added gives the amount of acid actually required in dissolving the $BaCO_3$ and which is thus the amount equivalent to it.

The amount of CO_2 present may then be determined by multiplying the number of cc. of N/10 HCl necessary to dissolve the precipitate by the factor 2.2 which gives the result in mg. of CO_2, since 1 cc. of N/10 HCl is equivalent to 2.2 mg. of CO_2, as shown by the following equations.

$$2\ HCl + BaCO_3 = BaCl_2 + H_2O + CO_2$$
$$44$$

2000 cc. N/HCl is equivalent to 44 g. CO_2
1 cc. N/1 Kcl is equivalent to .022g CO_2 =22 mg. CO_2
1 cc. N/10 HCl is equivalent to 2.2 mg. CO_2

With regard to the indicators used thymol blue, in the presence of $Ba(OH)_2$ gives a brilliant blue which changes

to a muddy green at the neutral point, gives a lemon yellow in slight excess, and pink in strong acid. The addition of brom phenol blue to the acid solution results in an orange color, which as the NaOH is added changes to a lemon yellow as the solution becomes slightly acid and to a light reddish purple at neutrality.

Before each respiration test the glass shells were thoroughly sterilized by heating in autoclave at 20 pounds pressure for 30 minutes and the other parts of the apparatus by dipping into a fifty percent alcoholic solution of mercuric chloride, of strength two to one thousand for two minutes and washing with sterile water blanks.

RELATION OF PRESOAK PERIOD TO CO_2 EVOLUTION.

Several tests were run to determine the effects of different lengths of presoak periods upon the rate of CO_2 production. The results of these tests are given in Table 7.

Table 7. Relation of time soaked to CO_2 evolved.

Test	Time soaked	Mg. CO_2 evolved	Average per period test
A	12 hrs	7.46	7.92
B	" "	8.36	
C	24 hrs.	11.88	11.99
D	" "	12.10	
E	48 hrs.	6.61	8.14
F	" "	9.68	

It is evident from this table that presoaking the seeds for 24 hours before running the respiration tests gave the best results as compared with presoaking for 12 to 48 hours respectively. Therefore, a presoak period of 24 hours was adopted for all the tests.

METHOD OF SEED STERILIZATION.

Two methods of seed sterilization were tried. In the calcium hypochlorite method, as developed by Wilson (32), 10 g. of commercial chloride of lime (titrating 28 percent chlorine) was mixed with 140 cc. of water and the supernatant liquid decanted off after about 10 minutes, the seeds being soaked in five times their volume of this solution for eight hours. In using mercuric chloride as the disinfectant the method suggested by Norton and Chen (20) was followed in which

the seeds are presoaked for 8 hours, as advised by Braun (4) to render the spores of pathogens on the seed coat more susceptible to the disinfectant and at the same time lessen seed injury, sterilized for 2 minutes in an alcoholic solution of mercuric chloride (2 grams to 1000 cc. of fifty percent alcohol), and washed three times by means of sterile water blanks. Since the seeds have already been presoaked for 24 hours in preparation for the respiration test it is evident that they are at once ready for sterilization without further soaking. The results of the tests with the two types of sterilization are given in Table 8.

Table 8. Effect of method of seed sterilization on amount of CO_2 evolved.

Method of sterilization	Mg. CO_2 evolved in 6 days (12 samples	
	Average per sample	Total
Calcium hypochlorite	10.6 mg.	127.6 mg.
Mercuric chloride	15.5 mg.	185.8 mg.

As shown by this table the mercuric chloride method of seed treatment had the least effect upon the viability of the seed, the seeds so treated producing an average of 15.5 mg. of CO_2 in six days, while an equal weight of seeds from the

same sample, treated by the calcium hypochlorite method, produced but 10.6 mg. of CO_2 under the same conditions. These results warranted the adoption of the mercuric chloride method of seed sterilization as being the most effective and at the same time the least detrimental to seed viability.

EFFECT OF SIZE OF SEED ON RESPIRATION.

In the work done on the respiration of germinating seeds the same weight of seed was used in each respiration test but, due to the great variation in the sizes of the seeds of the different varieties of sorghum, there was considerable variation in the number of seeds entering each trial. This brought up the question as to whether the amount of CO_2 production might not vary with the number of seeds present in a given weight of material. Would not several centers of respiration be more active than a smaller number representing larger seeds? Comparison was therefore made of the amount of CO_2 produced by two lots of seeds based on the number of seeds present in one half gram of material. One lot averaged 21.6 seeds to the 1/2 gram the number per sample varying from 14 to 26 and the other lot averaged 32 seeds, the number in this case varying from 28 to 39. In Table 9 is given the results of this test.

Table 9. Effect of seed size on respiration.

Number of sample	Number of seed per half gram	Average number of seeds	Mg. CO_2 per 6 days	Mg. CO_2 per seed per 6 days.
5	14-26	20	42.88	1.04
5	28-39	34	42.82	.684

While the data in this table is not extensive it seems to show that the average amount of CO_2 produced by a given weight of the larger seeds is practically the same as the amount produced by a like weight of smaller seeds and in keeping with this the CO_2 production per individual seed, as shown in the fourth column, is proportional to its size. This indicates the respiratory activity to be dependent upon the amount of material used rather than the number of seeds present and justifies the basing of respiration tests upon given masses of material.

EXPERIMENTAL DATA ON RESPIRATION.

Using the above experimental methods, respiration studies were carried on with the sorghum seeds to determine whether the reduced germination of injured seeds was entirely due to the direct effect of the mechanical injury or whether it might not be due, in part at least, to reduced viability as a result of the increased respiratory activity made possible by the break in the seed coat.

In all, approximately 100 respiration tests were made with different types of seed chosen from 25 samples. Tests from 11 representative samples are reported herein, three tests being made from each sample. The first test was made with whole or uninjured seed, the second with slightly injured or chipped seed, and the third with seed which were severely injured or badly broken, care being taken, however, to see that the embryo was present. The types of injured seed were separated out by aid of the binocular and are illustrated in Plate I.

A half gram of seed was used in each instance and the respirometers were kept in the dark in seed germinators where they were subjected to alternating temperatures of 30° C. for six hours and 20°C. for 18 hours as recommended in Department Circular 406 (Rules for Seed Testing) (31), the advantage of such manipulation being established by Harrington (13) in his work on the germination of seeds. The tests were run for six days, the CO_2 produced being determined and recorded at the end of each two-day period.

The results of these tests are given in Table 10 and are recorded in milligrams of CO_2 produced per gram of seed for each two-day period.

Table 10. CO_2 production by germinating sorghum seeds.

Sample Numbers	Milligrams CO_2 produced per gram of seeds per 2-day period.								
	Whole seed			Chipped seed			Broken seed		
	first period	second period	third period	first period	second period	third period	first period	second period	third period
67	11.60	12.82	18.96	7.92	11.80	14.42	4.96	5.34	7.38
68	14.38	19.18	23.52	8.20	13.08	15.70	4.18	5.64	6.20
72	8.80	13.20	17.60	6.40	9.96	12.66	4.40	6.16	8.36
92	7.48	14.52	51.40	5.22	6.98	11.34	3.50	6.04	7.20
96	4.80	16.56	16.12	3.96	13.20	12.32	3.96	3.08	7.14
97	8.30	11.20	15.58	6.02	10.20	12.24	3.04	2.02	2.02
100	8.62	13.80	14.44	7.04	13.20	13.64	2.60	3.46	3.02
104	12.64	18.74	22.20	9.40	11.52	16.68	3.90	4.88	5.88
105	13.20	14.52	15.85	10.52	12.94	15.36	6.52	4.88	4.88
64-A	12.00	15.10	19.48	8.40	12.96	16.82	4.50	4.82	6.52
64-B	13.08	14.40	17.16	8.92	11.96	15.78	4.08	5.06	7.00
Average	10.45	14.91	17.83	7.45	11.62	14.27	4.15	4.47	5.96

It is apparent from the foregoing table that regardless of whether the seed be uninjured, slightly injured or severely injured, the least amount of CO_2 was respired the first two-day period with an increase in the amount produced each succeeding period. However, with the whole and slightly injured seeds, the increase of the CO_2 production of the third period over the second is less than the increase of the second period over the first, indicating the rate of increase to be falling off and suggesting the proximity of the maximum respiratory activity as is apparent in the graph (Fig. 3) based on CO_2 production by periods. This is not evident in the case of the severely injured seeds, probably because of their slowness in attaining their maximum respiratory rate as a result of having lost a great part of their energy while in storage.

It is also apparent from Table 10 that in every instance the rate of respiration for the corresponding periods is the greatest in the case of the uninjured seeds, less for the slightly injured ones, and decidedly less for those severely injured. This effect of the different degrees of injury on the respiratory activity is better brought out in Table 11 in which a comparison is made of the total amounts of CO_2 produced during the entire six-day period by the three types of seed and also by the graph (Fig. 4) based on the average total CO_2 production.

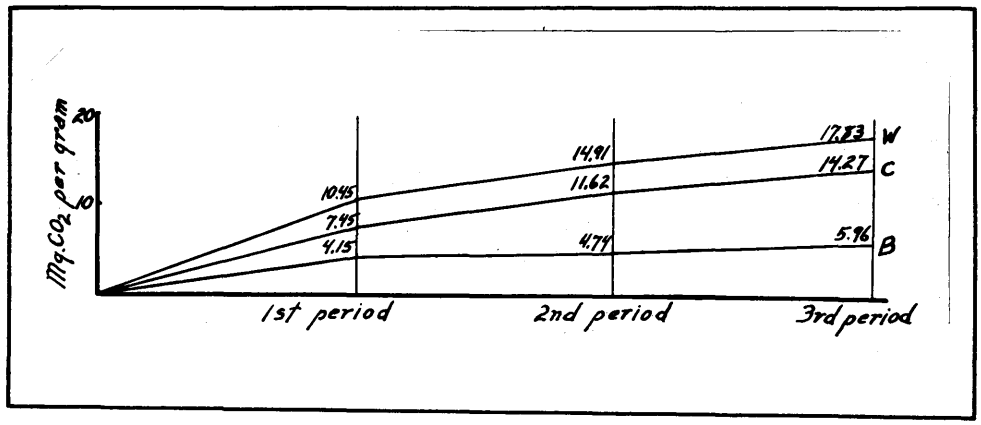

Fig. 3. Graph showing CO_2 production by periods.

W, whole seeds
C, chipped seeds
B, broken seeds

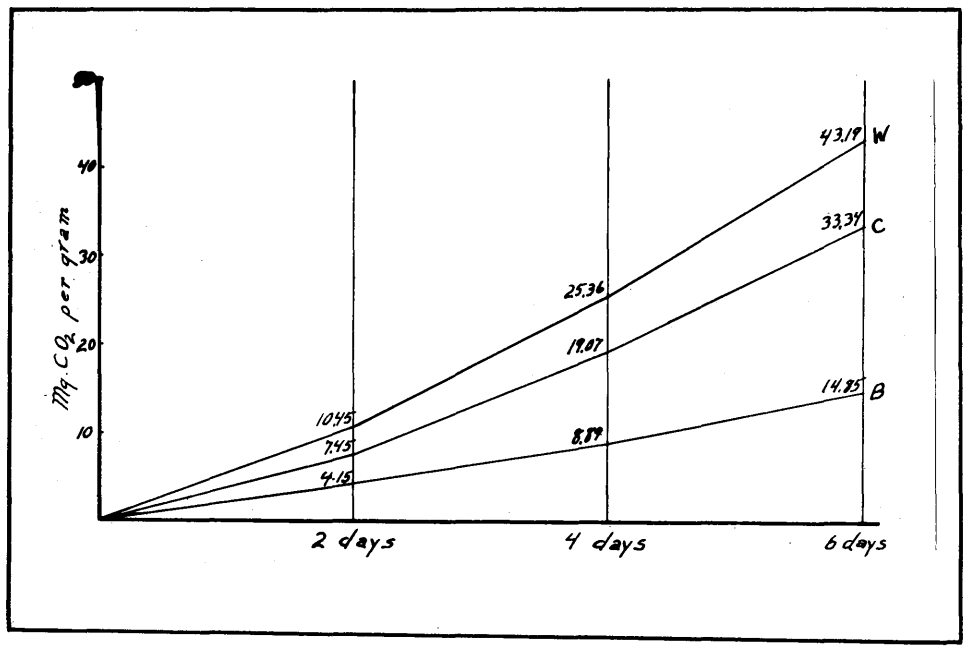

Fig. 4. Graph showing total CO_2 production from beginning of test to the end of each respective period:

W, whole seeds
C, chipped seeds
B, broken seeds

Table 11. Total amounts of CO_2 respired.

Sample Number	Total CO_2 in Mg. per gram seed in 6 days: Whole seeds	Chipped seeds	Broken seeds
67	43.38	34.14	32.68
68	57.08	36.98	15.84
72	39.60	29.02	18.92
92	37.40	23.54	16.74
96	37.48	29.48	14.18
97	34.88	28.46	7.08
100	36.86	33.88	9.08
104	33.58	37.60	14.66
105	43.56	38.83	16.28
64-A	46.58	38.18	15.84
64-B	44.64	36.66	16.14
Average	43.19	33.34	16.13

From the averages of the CO_2 production of the eleven representative samples composing this table it is seen that the broken seeds produced but 16.13 mg. CO_2 during the six-day period, the slightly injured seed 33.34 mg. CO_2, while in the same time, the whole seeds evolved 43.19 mg. CO_2. This lessened CO_2 production on the part of the injured seed suggests material lack of vitality.

Since it is generally considered that the rate of CO_2 production is the measure of respiratory activity and that the amount of CO_2 produced is a measure of viability, we may conclude from the above data that in the case of sorghum seeds mechanical injury may not only affect germination as a direct result of the break but also results in the depletion of the seed's energy or viability thru a process of

respiration while in storage and that, other factors being equal, the degree of loss in viability is proportional to the degree of severity of the injury.

This being true we may further conclude that the lowered germination of seed samples containing broken and injured seeds is due, in part at least, to the loss of viability of the seeds so injured.

THE RELATION OF ORGANISMS TO GERMINATION OF SORGHUM SEED

It is common knowledge that sorghum seed mold badly in the germinator. Whether this is also the case in the soil and whether certain soil fungi enter cracks in the seed and become parasitic or semi-parasitic, has never been determined. Likewise, little attention has been paid to the effect of the presence of bacterial organisms and nothing has been done to show whether the predominating pathogens are of internal or external origin.

That broken seeds are more susceptible to the attacks of organisms was ascertained by Hurd (17) in her work on the effects of seed injury of wheat and barley. She states that neither Penicillium nor Rhizopus ~~were~~ was ever found in healthy unbroken seeds germinating on blotters, but that if the seed coats were broken over the endosperm and the seeds germinated under non-sterile conditions, they were invariably badly attacked. Neither fungus, however, attacked seeds where the only

injury was a break in the coat over the embryo. She further concluded that the vitality of seeds is a factor in determining the ability of Penicillium and Rhizopus to attack them. Finnel (10) after working on the grain sorghums says : "The effect of low temperature is practically to prolong the time required for germination and thereby allow the attack of soil organisms to become destructive of the undeveloped or slowly developing embryo." Adams and Russell (1), in presenting the results of their study of Rhizopus infection of corn, state that seed infection with Rhizopus nigricans has been associated with certain symptoms and that germination tests carried on at Pennsylvania State College by C. R. Orton and E. L. Nixon proved various types of molds to be serious factors in preventing the development of the seedling. Norton and Chen (21) isolated a fungus which caused considerable damage to corn and proved to be carried internally by the seed. It was described as resembling Oospora verticilloides (Sacc.) and Cephalosporium sacchari. Edgerton and Kidder (9) , in studying the fungus infection of seed corn kernels and importance of germination tests in Lousiana, determined that many corn grains were internally infected with fungus parasites such as Diplodia zeae, Fusarium moniliform and Cephalosporium acremonium. Mans, Thomas and Adams (19) worked on internal fungi of seed corn in Delaware and found four prevalent fungous parasites: Cephalosporium sacchari (Butler), Fusarium monilimonium (Sheldon), Giberella saubinetti (Sacc.), and Diplodia zeae. Fusarium moniliform was considered as being the same as

Oospora verticilloides. They also found, internally in corn, species of the following genera: Aspergillus, Cladosporium Penicillium, Alternaria, Helminthosporium, Rhizopus, Spicaria Hormodendron, Torula, Chaetomium, Colletotrichum and several bacteria. Presence of internal organisms was not indicated by any uniform external symptoms. A review of literature by Chen (6) in connection with his work on the internal fungous parasites of agricultural seeds shows that internal parasites have been reported in the seeds of barley, beans, beets, bur clover, cabbage, clover, cotton, cucumber, darnel, evening primrose, flax, grasses, hollyhock, peas, rice, sweet corn, timothy, tomato and wheat. He states that organisms may be present as spores, as vegetative forms of bacteria, and as vegetative mycelium of fungi. In the various agricultural seeds worked with he found species of Cylindrophora, Alternaria, Fusarium, Macrosporium and Rhizopus. He also observed a fungus parasite of sweet corn similar to Oospora verticilloides, Rhizopus nigricans in seeds of rotten tomato, and a sterile fungus parasitic to wheat seedlings. Crawford (7) isolated from the interior of cotton seed Colletotrichum sp., Diplodia gossypii, three species of Fusarium, Cephalothecium sp. and Alternaria sp.

With regard to treatment of seeds Manns and Adams (19)

state that seed disinfection is not successful because of the manner of internal infection. Chen (6) advises seed selection from disease-free plants, germination tests and hot water and hot air treatments as means of control. Zundel (34) , working with wheat, and Briggs (5), doing similar work, showed, as have many other workers, that treatment with wet fungicides resulted in a high percent injury to germination. Finnel (10) in seeking a method of improving stands of grain sorghums by seed treatments found both copper carbonate and Bayer dusts effective, these treatments prolonging the resistance to fungous attack from three to five days. The earlier the planting the more urgent the necessity of treatment and this investigator secured as good a stand with Feterita with dust infection at 68°F. as with untreated seed at 82°C.

METHOD OF STUDYING ORGANISM INFECTION.

In order to determine the presence of organisms in sorghum seed and to find if they are superficial or internal, cultures were made of seed from a large number of samples. The cultures were made by plating the seed on Thaxter's potato agar in sterile Petri dishes and holding at a temperature 20 to 22°C. Six to 12 seeds were placed in each plate

and organism counts were made at the end of three and six days respectively. For the purpose of distinguishing the internal organisms from those on the outside of the seed it was necessary to make two cultures from each sample, one being of unsterilized seed (Plates IV to VI.) and the other after the external surfaces had been sterilized (Plates VII to X inc.).

Wilson's (32) calcium hypochlorite and Norton and Chen's (20) mercuric chloride methods of seed sterilization have already been described and compared in the work on respiration. Their respective effectiveness was again tried out in connection with the plate cultures and again the mercuric chloride method of treatment proved the more satisfactory.

In the case of the saccharine sorghums the glumes, which are usually persistent, were first removed from the seeds in order to insure the effectiveness of the sterilization process.

EXPERIMENTAL DATA ON ORGANISM INFECTION.

In all 242 cultures, using 1452 seeds, were made from the 141 samples of 1924 material and the gross results of these platings are shown in Table 12 in which it will be noted that the degree of infection is recorded with regard to the percent of samples infected with the various types of organisms and also with regard to the percent of individual seeds infected.

Plate IV.

Showing Petri dish culture of unsterilized seeds infected with bacteria.

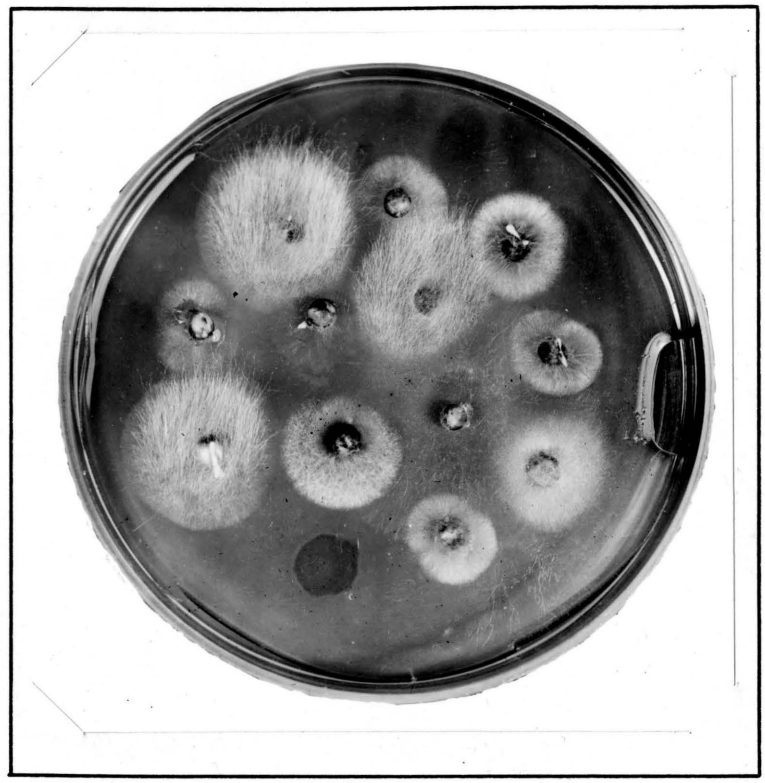

Plate V.

Showing Petri dish culture of unsterilized seeds infected with fungi.

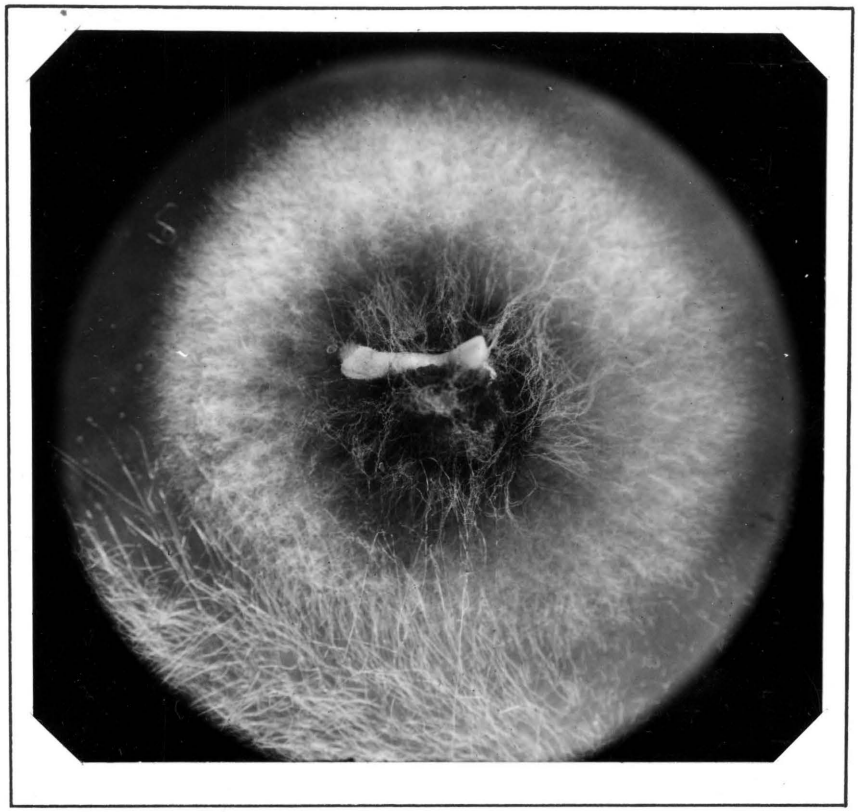

Plate VI.

Showing germinating unsterilized seed overgrown with fungus.

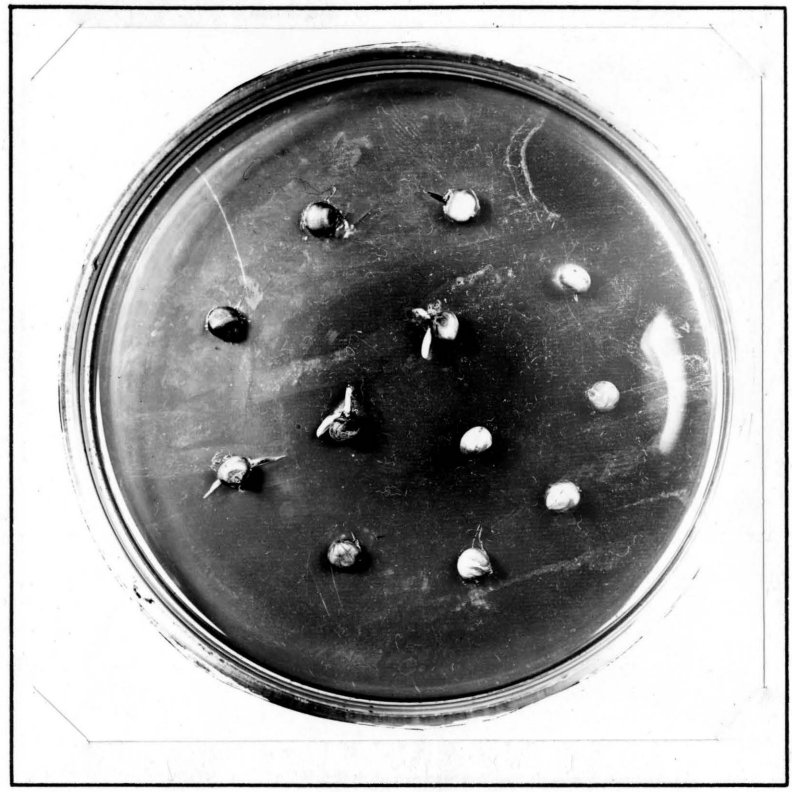

Plate VII.

Showing Petri dish culture of
sterilized seeds after
three days; no infection

Plate VIII.

Showing Petri dish culture of sterilized seeds after six days;no infection

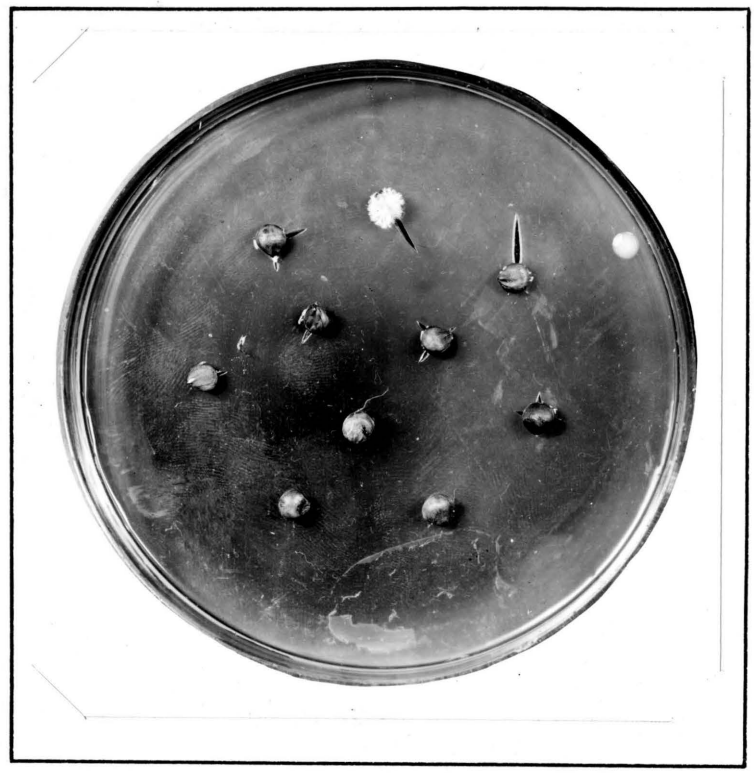

Plate IX.

Showing Petri dish culture of sterilized seeds after three days; one internal fungous infection.

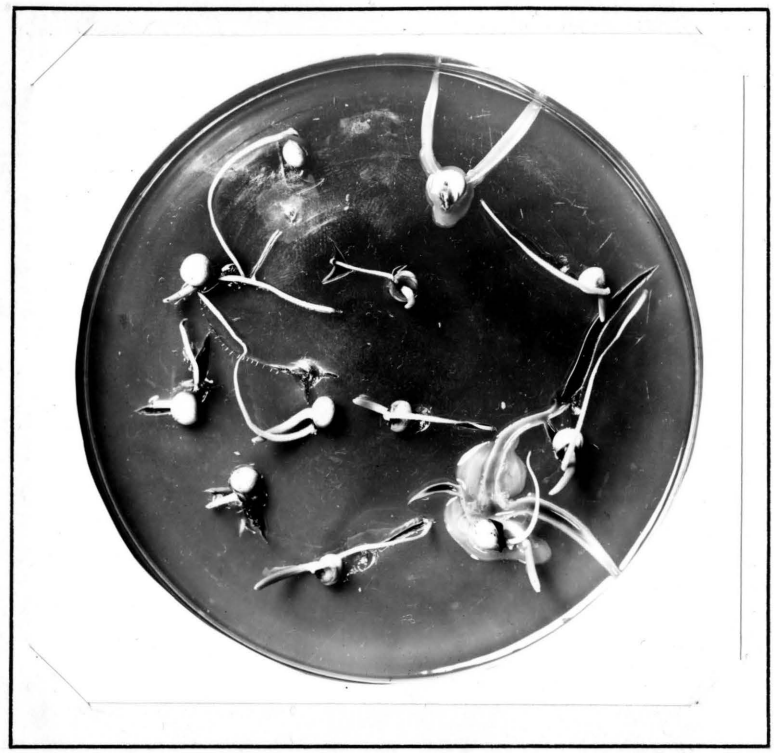

Plate X.

Showing Petri dish culture of sterilized seeds after six days; two internal bacterial infections.

Table 12. Degree of infection in sorghum seed.

	Percent Infection	
	Samples	Seeds
External fungi	98	74
" bacteria	90	64
Internal fungi	34	10
" bacteria	80	38

From the study of this table it appears that practically all of the samples were infected with external organisms with a somewhat greater prevalence of fungi than bacteria. The same relation holds with regard to the infection of the individual seeds althouth the degree of infection is considerably less.

The number of samples infected with internal organisms was noticeably smaller, especially in the case of the internal fungi which were present in only 34 percent of the samples while internal bacteria were found in 80 percent, thus showing a much greater prevalence of internal bacteria than fungi. This same relation holds in the case of the individual seeds, 10 percent having internal fungi and over three times as many or 38 percent having internal bacteria.

In considering the effect the presence of glumes may have on the degree of infection, Table 13 is presented, in which a comparison of the infection of grain and saccharine sorghums is made.

Table 13. Comparison of degree of infection of grain and saccharine sorghums.

	Grain sorghums			Saccharine sorghums		
	Number of samples	Percent Infection: Samples	Seeds	Number of samples	Percent Infection: Samples	Seeds
External fungi	48	100	86	93	97	68
" bacteria	48	86	50	93	92	72
Internal fungi	48	24	7	93	39	11
" bacteria	48	76	36	93	84	40

In the above table it may be seen that there is little difference in the two types of sorghum seeds as regards the percent of samples infected with external organisms but that internal pathogens are somewhat more prevalent in the saccharine than in the grain types, this being especially marked in the case of the fungi.

With regard to the individual seed infection we find a greater variation in conditions. The saccharine sorghums show a lower infection of external fungi as compared to the grain sorghums but a higher degree of infection with both internal and external bacteria and internal fungi. In making a comparison of the two types of sorghum seed with regard to their prevailing organisms it is noticeable that in the saccharine sorghums there is a higher percent bacterial infection, both internal and external, than fungous infection. This is also true of the grain sorghums so far as internal

pathogens are concerned, the bacteria again predominating, but with regard to their external infection the reverse condition obtains, the percent of external fungi greatly exceeding the percent of external bacteria. Although it appears from this that the saccharine sorghums proved to be somewhat more severely infected with internal organisms, both fungous and bacterial, than the grain sorghums, and also more severely infected with external bacteria, there is hardly enough data to warrant drawing any conclusions as to whether this fact may account for their lowered germination in the laboratory, although it is very possible that such may be the case.

The bacteria found in the above platings were not determined. Species of the following genera of fungi, however, were as follows in order of prevalence:

External: Rhizopus, Alternaria, Penicillium, Fusarium, Aspergillus, Trichoderma, Macrosporium, Phoma, Botrytus.

Internal: Alternaria, Fusarium, Rhizopus, Penicillium Aspergillus, Cephalosporium, Trichoderma. The following species were determined: Aspergillus niger, Rhizopus nigricans, Fusarium moniliform, Trichoderma lignorum.

Seeds that failed to germinate in the soil in the greenhouse were dug up and a hundred such seeds plated on agar without sterilization. After two days they were overwhelmed with

with bacteria and in four days Fusarium sp., Cephalosporium sp., and Rhizopus sp. were evident.

Another group of these seeds ~~were~~ was washed with brush and water, sterilized externally with mercuric chloride by the method described earlier in this paper, and likewise plated. Out of 125 seeds, 109 proved to be infected with bacteria, 55 with Fusarium sp., and 24 with Rhizopus sp.

SEED TREATMENT.

As has already been shown (Table 2.) the average field germination of the sorghum seed is far below the average laboratory germination of the same samples. Of interest in this connection it may also be said that in the field tests every ungerminated seed that was recovered proved to be infected either with fungi or with bacteria and in many cases with both as shown in Plate XI. To what extent these organisms are instrumental in causing the non-germination of the seeds or whether they are largely saprophytic on seeds already dead or greatly reduced in vitality, is at present, largely a matter of conjecture, although Hurd (17) found that on the germinator certain fungi attacked the seed only when the seed coat was broken and that the ability to attack depended largely upon the reduced vitality of the seed. Table 3 brings out the fact that although the grain sorghums have the higher germination in the laboratory, as compared to the saccharine

sorghums, the latter give the better results under field conditions. This indicates the presence of certain unfavorable factors in the field against which the persistent hull of the saccharine types of sorghums may offer some degree of protection. The above facts, together with our knowledge of the existence, on the seed and in the soil, of various pathogenic organisms, suggests the advisability of seed treatment, and justifies the expenditure of considerable time and effort in ascertaining the most successful methods.

DESCRIPTION OF SEED TREATMENTS.

The effect of various types of seed treatments under field conditions was tried out for two successive years. Fifty samples of 1924 seeds were used the first year and included both grain and saccharine types of sorghums. Twenty-four samples of 1925 seeds were used the following year and included only grain sorghums. In each test half the samples chosen were of marked low germination and the other half of exceptionally high germination. In the first trial copper carbonate, copper stearate, mercuric chloride, and hot formalin treatments were used.

The copper carbonate was applied in powder form at the rate of 3 ounces to the bushel. The copper stearate was applied

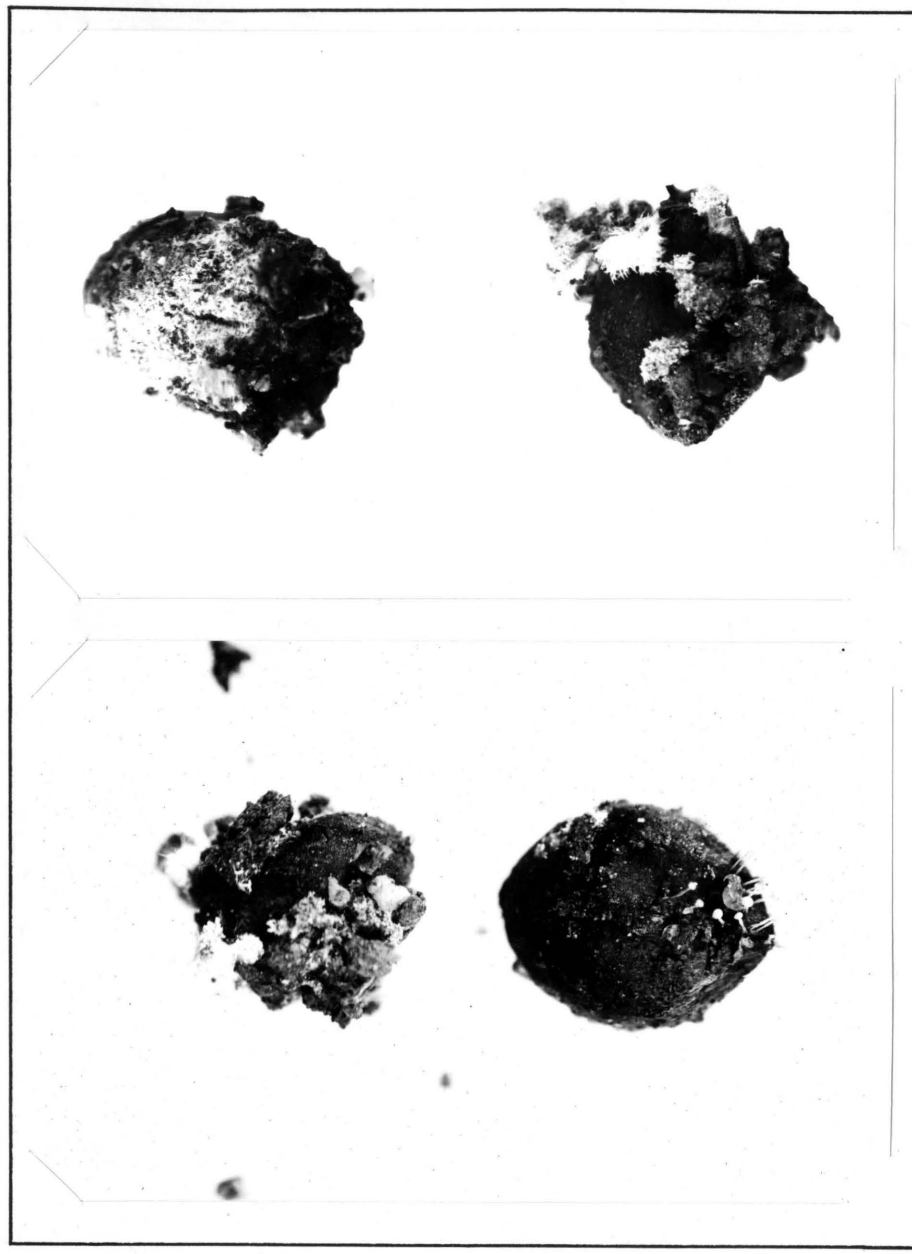

Plate XI.

Showing seeds removed from soil
infected with soil organisms.

in a similar manner at the rate of 6 ounces to the bushel. The mercuric chloride solution was made by dissolving 1 gram of mercuric chloride in 1000 cc. of 50 percent alcohol, the seeds being immersed for 30 seconds. The formalin solution was prepared in the proportions of 1 pint of the commercial produce to 40 gallons of water (1:320) and held at 51° C., the seeds in this case being submerged and removed as quickly as was consistent with the thorough wetting of the seed coat.

In the second year's trial the same treatments were used except that Bordeaux paste was used in place of copper stearate, the mercuric chloride soaking was lengthened to two minutes and the formalin solution was used cold, the seeds being immersed for a full ten minutes.

The Bordeaux paste was prepared in the same manner as Bordeaux spray mixture except that the amoung of water was such as to give the mixture a paste-like consistency, thereby making possible the thorough coating of each individual seed.

The seeds were planted in rows a foot apart and eight feet long, 100 seeds being planted to the row. A check row of untreated seed was also planted along with the treated seeds from each sample.

EXPERIMENTAL DATA ON SEED TREATMENTS.

The results of these experiments are given in Tables 22, 23, and 24 and their accompanying histograms (Figs. 5, 6, and 7).

Tables 22 and 23 and their histograms (Figs. 5 and 6) show the comparative effects of the various treatments on the germination of high and low germinating seeds of the years 1924 and 1925 respectively.

Table 22. Effect of seed treatments on high and low germinating seeds (50 samples 1924 seed).

	Laboratory germination.	Field test germination.				
		Check	Copper carbonate	Copper stearate	$HgCl_2$	Formalin.
High germination	88.0	73.5	79.6	75.5	73.2	70.4
Low germination	54.0	26.4	32.9	29.8	22.8	23.8
Average	62.2	35.8	42.2	38.9	34.9	33.1

Table 23. Effect of seed treatments on high and low germinating seed (24 samples 1925 seed).

	Laboratory germination	Field test germination				
		Check	Copper carbonate	Bordeaux paste	$HgCl_2$	Formalin.
High germination	93.0	58.0	64.0	63.1	37.0	45.2
Low germination	36.0	12.2	16.8	16.0	7.0	6.4
Average	64.5	34.1	40.4	38.0	22.0	28.7

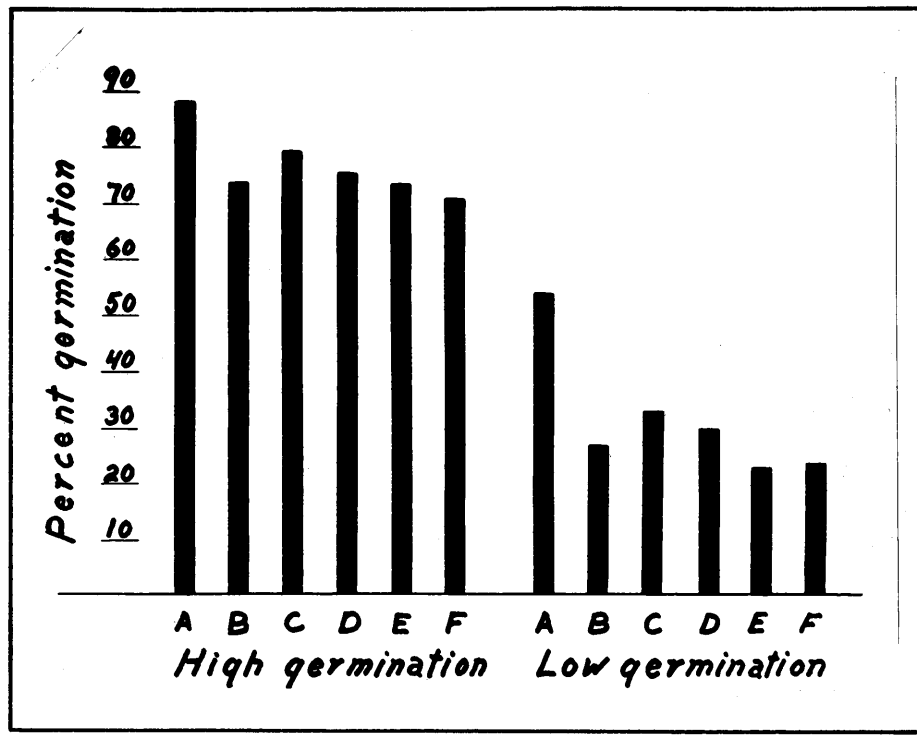

Fig. 5. Histogram showing the effect of seed treatment on the field germination of high and low germinating 1924 seed.

A, laboratory
B, check, untreated
C, copper carbonate
D, copper stearate
E, mercuric chloride
F, formalin, hot

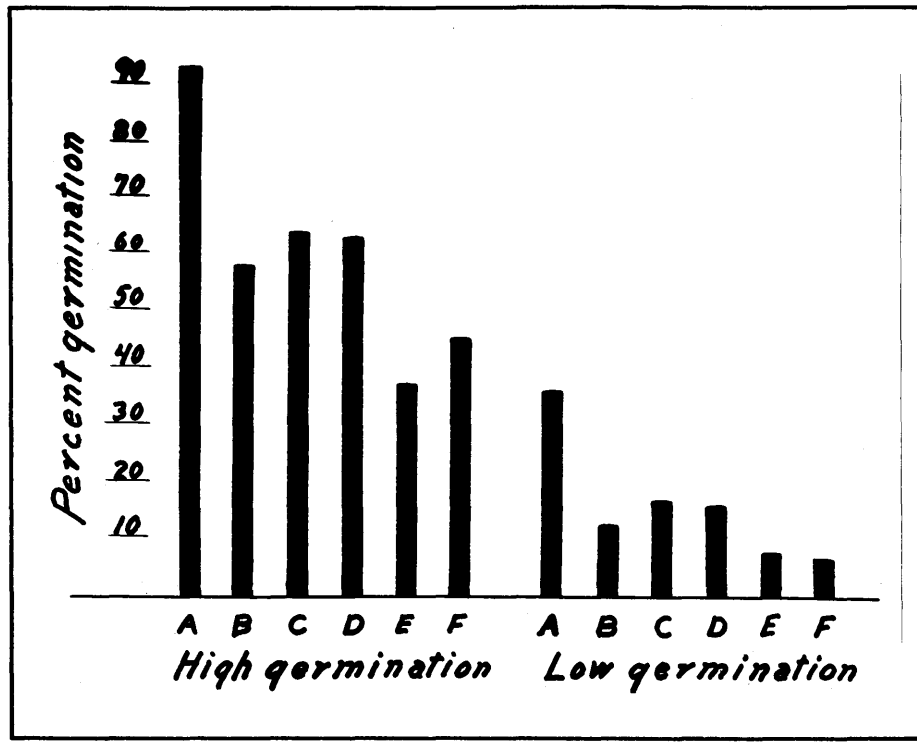

Fig. 6. Histogram showing the effect of seed treatment on the germination of high and low germinating 1925 seed.

A, laboratory
B, check, untreated
C, copper carbonate
D, Bordeaux paste
E, mercuric chloride
F, formalin, cold

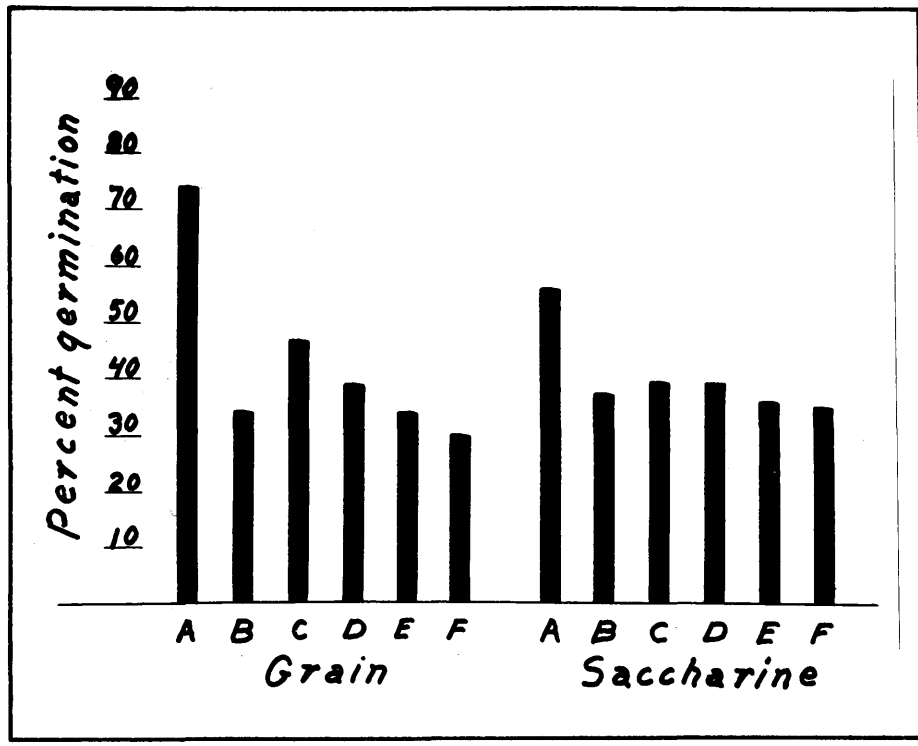

Fig. 7. Histogram showing the effect of seed treatment on the germination of grain and saccharine sorghums, 1924 seed.

A, laboratory
B, check, untreated
C, copper carbonate
D, copper stearate
E, mercuric chloride
F, formalin, hot.

Table 24 and histogram (Fig. 7) show the comparative effects of the treatments on the germination of grain and saccharine types of sorghums of the year 1924 only.

Table 24. Effect of seed treatments on grain and saccharine types of sorghums (50 samples 1924 seed).

	Laboratory germination	Field test germination				
		Check	Copper carbonate	Copper stearate	$HgCl_2$	Formalin.
Grain	74.0	34.0	46.7	38.7	33.6	30.0
Saccharine	56.0	37.0	39.2	39.1	35.7	35.0
Average	62.2	35.8	42.2	38.9	34.9	33.1

It is evident from a study of the data in the above tables that the germination of the sorghum seeds may be considerably influenced in the field by the use of seed treatments and that similar results obtain whether the seeds in question be of high or low average germination or of the grain or saccharine type of sorghum. However, it is apparent from Table 24 and histogram (Fig. 6) that the grain sorghums were benefited to a much greater extent than were the saccharines, copper carbonate, for example, increasing the germination of the grain sorghums from 34 percent to 46.7 percent, an increase of 12.7 percent or approximately one-third, while in the case of the saccharine sorghums the germination was raised from 3.7 percent to 39.2 percent, an increase of but 2.2 percent.

A comparison of the effectiveness of the different treatments is made in Table 25, which is based upon the percent change in germination resulting from their use.

Table 25. Percent of change in germination resulting from seed treatment.

	Percent of change in germination.					
	Check	Copper carbonate	Copper stearate	Bordeaux paste	$HgCl_2$	Formalin
1924 seed	0	+18.0	+9.0		-2.5	-7.5
1925 seed	0	+18.5		+12.0	-35.0	-26.0
Average	0	+18.3	+9.0	+12.0	-18.7	-16.7

From Table 25 and from the preceding tables and histograms concerning seed treatment, it is also evident that of the treatments tried, the one using powdered copper carbonate proved the most beneficial, increasing the average germination of all samples tested 18.3 percent. Bordeaux paste ranked next with an increased germination of 12 percent and the copper stearate powder next with an increase of 9 percent. Both wet treatments proved to be injurious to the seed, the mercuric chloride decreasing the germination 18.7 percent and the formalin decreasing it 16.7 percent. In both cases the increasing of the length of time of immersion resulted in increased injury, as shown by a comparison of the second year's test (Table 23) with that of the first year (Table 22).

The effectiveness of the dust treatments and the Bordeaux paste is no doubt due to the lasting qualities of this type of treatment. The protective covering remains active upon the

seed for a considerable period after the seed is placed in the soil and thus prevents infection from soil organisms. Other types of treatment merely serve to kill the organism on the seed at the time of treatment but offer no protection to organisms that may be met with later in the soil. Such being the case it is evident that the percent increase in germination resulting from efficient dust treatments is an index to the amount of injury resulting from the attack of external soil organisms.

From these results it is apparent that sorghum seed is benefited by seed treatment and that the grain sorghums are benefited to a much greater extent than the saccharine types. Furthermore, the degree of benefit derived warrants, at least in the case of the grain sorghums, the use of such treatments under actual farming practices, and the recommendation of powdered copper carbonate in this connection, not only because of its greater effectiveness as shown by the above tests but also because of its generally proven cheapness and ease of application. With regard to the saccharine sorghums it appears that the protection apparently afforded by the persistent glumes is not sufficiently augmented by seed treatments to warrant their use, and that under soil conditions favorable to the development of injurious organisms the saccharines are preferable to untreated grain sorghums.

CONCLUSIONS.

Sorghum seeds in general have a low percentage germination and Colorado grown sorghums are no exception to the rule the average laboratory germination, over a five-year period, being but 71 percent. Field tests showed the field germination to be but little more than half the laboratory germination and under adverse conditions are probably even lower. Since there are around 400,000 acres planted to this crop annually in the state it is evident that this characteristic low germination results in considerable loss, not only in the value of the seed planted but even more so in the decreased yield resulting from poor stands and shortened growing season following replanting.

This typical low germination of the sorghum is apparently due to the concerted action of several different factors. Among the most important of these are mechanical injury, respiration of seeds in storage, and the attack of soil organisms. Observations made also indicate that the degree of maturity has an important bearing upon the viability of the sorghum seeds, and more conclusive evidence should be worked up concerning the effect of this factor.

With regard to the varying amounts of broken seeds present in the different samples a study was made of the structure of the seed coats of different varieties of sorghums and the results indicated the existence of a certain relationship between this structure and the tendency of the seed to breakage. Further work is essential, however, before definite conclusions can be drawn regarding this relation.

It is apparent that the effect of seed injury upon germination is in general proportional to the degree of the injury while the location of the break appears to make but little if any difference.

That loss of germinating power is not only due to the direct effect of mechanical injury but may also result from loss of viability thru increased respiration in storage is evident from the respiration tests made with whole, scratched and broken seeds, the results of which clearly show such loss of viability and consequent decrease in germination power to again be proportionate to the degree of the injury.

In the respiration studied it was evident that the CO_2 production was porportional to the mass of seed used and independent of the size of the individual seeds making up the mass.

The respiration rate of the germinating seeds was found to be comparatively low at first increasing rapidly up to the sixth day soon after which it apparently reached its maximum.

This suggests the necessity of seed protection against the attack of soil organiams during the early stages of germination and before the young embryo has attained a vigorous state of growth.

Both bacteria and fungi were found to be important factors in reducing germination and they may be of internal or external origin. However, of the two types it was evident that the external organisms, especially those existing in the soil , were the more deleterious.

The saccharine sorghums are much less effected by soil conditions than the grain sorghums and also much less effected by seed treatment, indicating the protective nature of the persistant hull.

Of the various seed treatments tried the dry powdered types proved the most beneficial, due to the protective coating remaining on the seed when planted and preventing infection from soil organisms. In this respect the copper carbonate proved the most effective and gave the best results.

The wet treatments gave a lower germination than did the untreated seeds due in part to the deleterious effect of this type of treatment on the seed and in part to the fact that no protection was afforded against the soil organisms.

Thus under field conditions, it is evident that the use of copper carbonate as a seed treatment is advisable in the

case of the grain sorghums in order to insure a stand and prevent losses that might otherwise occur. So far as the saccharine sorghums are concerned the seed treatments tried appear to have but little value. However, in damp, cold soils favorable to organisms and unfavorable to sorghum seeds the saccharine sorghums , due to their greater immunity to external infection, are preferable to untreated grain types, in so far as germination alone is considered.

SUMMARY

(1) The average germination of sorghum seed is low, the laboratory average of Colorado-grown sorghums over a period of five years being but 71 percent, while in the field the germination is little better than half that in the laboratory.

(2) Low germination results in a great loss in the state especially when replanting is necessitated as the result of poor stands.

(3) The germination of the saccharine sorghums is less effected by field conditions than is the germination of the grain sorghums.

(4) Mechanical injury is very evident in a majority of the samples of sorghum seeds.

(5) The structure of the seed coat appears to bear a relation to the degree of prevalence of seed injury.

(6) Mechanical injury lowers the germination of the sorghum seeds and in general does so in proportion to the degree of the injury.

(7) Injury over the embryo was not shown to have any more deleterious effect than injury elsewhere.

(8) The direct effect of pure mechanical breakage is accountable for only a part of the lowered germination.

(9) A simple type of respirometer was devised for use in the respiration studies.

(10) A presoak period of 24 hours proved to be the most desirable in the respiration tests.

(11) Chen's mercuric chloride method of seed sterilization was found to be more desirable than the calcium hypochlorite method in the respiration tests.

(12) The size of the seed did not bear any relation to the amount of CO_2 produced by a given mass of seed.

(13) The rate of respiration constantly increased during the six-day test, the curve of CO_2 production indicating the maximum rate to occur shortly after the sixth day.

(14) The total amount of CO_2 produced in the six-day tests was greatest in the case of the whole seed, less for the chipped seeds and much less for the broken seeds, indicating the loss of viability to be due to increased respiration in storage and proportionate to the degree of the injury.

(15) There was a much greater prevalence of external organisms than internal.

(16) External fungi were more prevalent than external bacteria.

(17) Internal bacteria were more prevalent than internal fungi.

(18) The saccharine sorghums proved to be somewhat more severely infected with internal organisms and also with external bacteria than the grain sorghums.

(19) The following external fungi were determined, in order of prevalence: Rhizopus nigricans, Alternaria spl,

Penicillium sp., Fusarium moniliform, Aspergillus niger, Macrosporium sp., Phoma sp., Botrytus sp., and Trichoderma lignorum.

(20) The following internal fungi were determined, in order of prevalence: Alternaria sp., Fusarium moniliform, Rhizopus nigricans, Penicillium sp., Aspergillus niger, Aspergillus sp., Cephalosporium sp. and Agrostalagmus sp.

(21) Seeds failing to germinate in the soil, when removed and plated, were found to be severely infected with bacteria and fungi, both internally and externally.

(22) Copper carbonate, copper stearate, Bordeaux paste, mercuric chloride, and hot and cold formalin were tried in seed treatment tests.

(23) The treatment using copper carbonate proved the most desirable.

(24) The wet treatments proved to be injurious to the seed.

(25) Seed treatments had considerable effect on the germination of the grain sorghums but very little on the saccharine types.

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