THESIS

THE PHYSIOLOGY OF BROKEN AND ABNORMAL WHEAT SEEDS

Submitted by
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May 1, 1928

This is to certify that Miss Mildred E. Lyon has translated for me assigned passages of technical French bearing upon her graduate Botanical work.

Respectfully,

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THIS THESIS HAS BEEN READ
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THE PHYSIOLOGY OF BROKEN AND ABNORMAL WHEAT SEEDS.

Mildred E. Lyon.

INTRODUCTION.

In the analysis of cereal seeds received for purity test at the State Seed Laboratory there is constantly present a varying percentage of broken and abnormal seed. It is generally thought that the germinating capacity of such seed is seriously impaired, but to what degree is not known.

The disposal of the broken seeds presents a difficult problem to the seed analyst in making purity analyses. Under the present rules for seed testing formulated and adopted by the Association of Official Seed Analysts of North America (44) many of these pieces of seed are placed in the pure seed class by virtue of the fact that each consists of more than half of a seed.

This ruling does not take into account the fact that in many of the pieces only a part of the embryo remains and obviously cannot germinate. Some of the broken seeds germinate when placed under the proper conditions though the shoot and root development of such seeds is practically always abnormal. It has been demonstrated that these seedlings, if they grow into plants at all, do not usually develop as normal seedlings do.

It is not definitely known to just what extent the seed must be injured to affect the resulting plant, that is, whether all breaks are detrimental in proportion to their size and location or whether the smaller breaks are of no consequence.

It is also not known whether such breaks result in an injury of a purely physical nature in which the vegetative organs alone are affected, in a loss of energy resulting from changes in the respiratory rate during storage or in a lowering of germination induced by fungal infection.

As a means of solving these problems a series of experiments on germination and respiration was undertaken in which wheat was used as a representative cereal crop.

While examining various lots of wheat for injured seed an interesting phenomenon, heretofore unnoted, was repeatedly found. In all lots of seed examined there occurred abnormally developed seed having normal endosperm but no embryo. As it is generally believed that the epithelial layer of the embryo is the seat of respiratory and enzymatic activity, the occurrence of embryoless seeds offered an opportunity to test this theory without excising the embryo. A study of the occurrence and behavior of these embryoless seeds is therefore presented in the latter part of this thesis.

PART I. STUDIES ON BROKEN SEED.

Introduction - The detrimental effect of threshing on cereals has been noted by various workers. Ringlemann (37) makes the statement that despite careful regulation of the threshing machine there are always some cracked seeds, occasioning, as a result,a loss or retardation in their germination.

The work presented in this paper was undertaken with the purpose of determining the nature of the injury received by wheat during the threshing process and the degree to which various types of breaks injure the seed. There are three ways in which mechanical injury may operate to cause lowered germination. These factors may work independently or collectively and it is quite probable that their effects upon the seed are coexistent.

Injury may be attributed to increased respiration caused by breakage, to the activity of fungi, entering by means of the break, or to the mechanical injury caused by the break itself.

The following discussion comprises a study of the effect of respiration and mechanical injury on germination of broken wheat seeds.

Materials.- The broken wheat used in these studies was selected from some 200 machine threshed samples received at the Colorado State Seed Laboratory for purity and germination tests. These lots of wheat were harvested from the crops of 1924, 1925, 1926 and 1927, and included all of the varieties of spring and winter wheat commonly grown in this state.

The types of broken seed which were selected for experimentation were those which are of the most general occurrence in wheat as the result of threshing. The seeds used were all more than half a seed and were grouped as follows:

1. Seed showing slight injury at embryo end
2. Seed showing severe injury at embryo end
3. Three-fourths seed, the injury at the brush end
4. Seed split lengthwise.

In Figures 1 to 4 are shown typical seeds of these types. It is obvious that the split seed is the most severely injured of the four types while the slightly injured seed which has a very small part of the proximal end broken away is least injured. These two types constitute approximately 90 per cent of the broken seeds of practically all lots of wheat. The severe injury at the

Fig. 1. Wheat seeds showing slight injury at embryo end.

Fig. 2. Wheat seeds showing severe injury at embryo end.

Fig. 3. Wheat seeds showing three-fourths injury at brush end.

Fig. 4. Wheat seeds split lengthwise.

embryo end doubtless has a deleterious effect on the development of the embryo while the three-fourths seed with a piece broken from the brush end probably suffers no ill effects other than that which might be attendant upon the loss of food material.

For each experiment with injured seed a check test was made with whole seeds selected from the same lot. These were carefully chosen with the aid of a Hasting's triplet 7X lens to insure their soundness. The broken seeds were selected and grouped in the same manner.

Injury as Affecting Respiration in Cracked Wheat Seed.- It has been observed by Harrington (21) and Tashiro (42) that injury to the seed coat results in increased respiratory activity in seeds. In seed cracked or broken in threshing this increased respiratory activity may be a controlling factor affecting the germinating power.

In discussing respiration Palladin (34) states that in seed plants the destruction of carbohydrates furnishes energy for respiration while Haas and Hill (19) call attention to the fact that the continuance of respiration ultimately depends upon adequate food supplies. Upon consideration of these statements it is logical to assume that a seed in which the reserve food material has been broken away, specifically, the split and three-fourths types of broken seed, will lose its germinating capacity much sooner than a whole seed.

In like manner, since injury stimulates respiration, we may be justified in assuming that over a period of time the respiration of a seed may be increased by wounding to the point where the stored food or the vitality of the embryo is seriously depleted. In connection with this theory it is a generally accepted fact that the heat of a chemical reaction depends upon the initial and final products and furthermore, that the total heat evolved is the same by whatever method the final products are attained, that is, whether the reaction proceeds slowly or rapidly. The wheat seed which contains a given amount of potential energy may be considered in the light of this principle. If this self- contained energy is dissipated more rapidly through an increased respiration rate than is the energy of a seed in which respiration proceeds normally vital activity will be more quickly exhausted. Respiration is a transformation of energy and consequently a premature death of the seed results when respiration is accelerated over a period of time.

It is doubtless true that this increased output of energy accompanying increased respiration in stored broken seeds is lost in the form of heat. Bailey and Gurjar (7) have made an extensive study of respiration in stored wheat, and find spontaneous heating in bulk grain to be a direct effect of respiration.

The relation of CO_2 evolution to vital activity of living organisms during the respiratory process, has long been recognized. Osterhout (33) concludes that respiration is the most fundamental of all life processes. Growth, reproduction, motion, irritability and constructive metabolism may cease as in the resting seed, yet life may go on for many years. When destructive metabolism ceases life is at an end. A resting seed furnishes an excellent application of this test. As long as it is alive it produces CO_2 but when this evolution ceases it is dead. Tashiro (42) states that respiration is a necessary condition for living processes and an unfailing sign of life, and that a more certain criterion than the process of CO_2 evolution itself, is an increase in respiration upon wounding.

Respiratory intensity as an index of longevity of seeds has been used by a number of workers in this field. Pierce (36) found that the heat given off by germinating peas decreased with age. Shull (39) working on the oxygen minimum of Xanthium seeds demonstrated that a slow progressive deterioration takes place in the seeds after the coats have become permeable to oxygen which leads to complete loss of germinating power. After conducting intensive studies on longevity of seeds Duvel (17) reported that certain chemical changes take place during respiration that exert a marked influence on the viability of seeds. The protoplasm of the individual cells gradually becomes disorganized and the energy stored within the seed is gradually dissipated, vital processes are destroyed and life becomes extinct. A different view entirely is taken by Blackman (8) on this same subject. He states that loss of germinating power after many years cannot be attributed to loss of material in respiration. Respiration is so small at low temperatures that a seed in a thousand years would not lose a considerable part of its material. The loss of it is doubtless due to slow changes in the proteins, probably to denaturing of the protein.

From a study of such conclusions as have just been given it is evident that any alteration in the mechanism of respiration, a process so definitely allied with vitality, must certainly have its ultimate effect upon the functions of the seed. So with broken seeds this injury to the living tissue results in an increased rate of respiration.

Tashiro (42) has shown that wheat seeds stimulated by injury display a marked acceleration in respiration.

Johnstone (25) working with sweet potatoes concludes that boring increases the respiration rate to a marked extent. Harrington (21) finds mechanical injury of various types beneficial in forcing the germination of freshly harvested cereals, he attributes this effect to an increase or alteration in the nature of respiration resulting from the injury. Bailey (6) observed that lots of corn containing high percentages of cracked and broken seeds respired at a much higher rate than did lots more nearly made up of whole seeds. Shull (40) working with semi-permeable seeds and Atwood (5) on the germination of _Avena fatua_ found that injury to the seed coat permitted a rapid passage of oxygen. In like manner Harrington (22) concludes that the removal of seed coats in dormant apple seeds caused an increase in the rate of respiration as well as germination.

Methods Used in Respiration Tests - In order to determine any possible correlation between increased respiration due to breaking and lowered germinability of seed, studies were made of the evolution of CO_2 from seeds representative of the previously described types of breaks as compared with that from whole seeds. Briefly stated the method used in these experiments involves the absorption of CO_2 by a saturated solution of barium hydroxide, and the measurement of the amount of CO_2 absorbed over a period of time by titration.

Description of apparatus and methods used in measuring respiration.- The respirometer used in these experiments is of extremely simple construction, a modification of that designed by Harrington and Crocker (23) for small objects such as seeds.' The advantage of this apparatus lies in its simplicity which permits the construction and the use of numerous respirometers at the same time. This insures identical experimental conditions for each type of seed in any one experiment. The apparatus (Figure 5) consists of a shell vial (A) 9 cm. deep by 3 cm. in diameter a No. 7 rubber stopper (B) a copper wire (C) and a paper cup (D) 2 cm. deep with perforations at the base.

The seed cup is suspended from the stopper by means of the wire. Both cup and wire are coated with paraffin to render the cup impervious to water. A saturated solution of CO_2 -free barium hydroxide is measured into the shell vial by means of the apparatus shown in Figure 6. This consists of three wash bottles (A) containing NaOH solution, a large bottle of saturated barium hydroxide solution (B) with an inlet (C) from the wash bottle and an outlet in the form of a

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'Thornton, Bruce J. Factors causing low germination in sorghum seed. Unpublished thesis, Colo. Agric. College 1927.

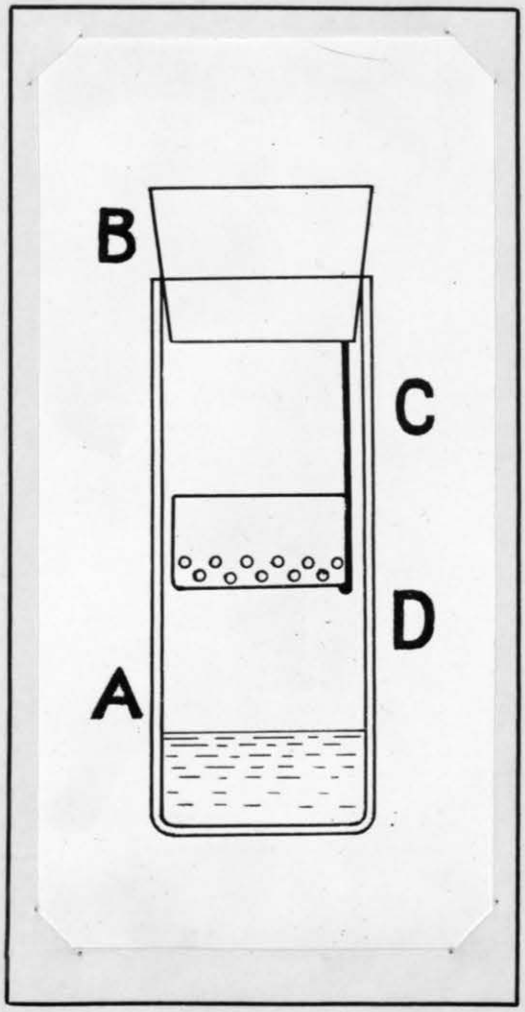

Fig. 5. Respirometer used in conducting respiration tests.

A. Pyrex shell vial
B. Rubber stopper
C. Copper wire
D. perforated, waxed paper cup.

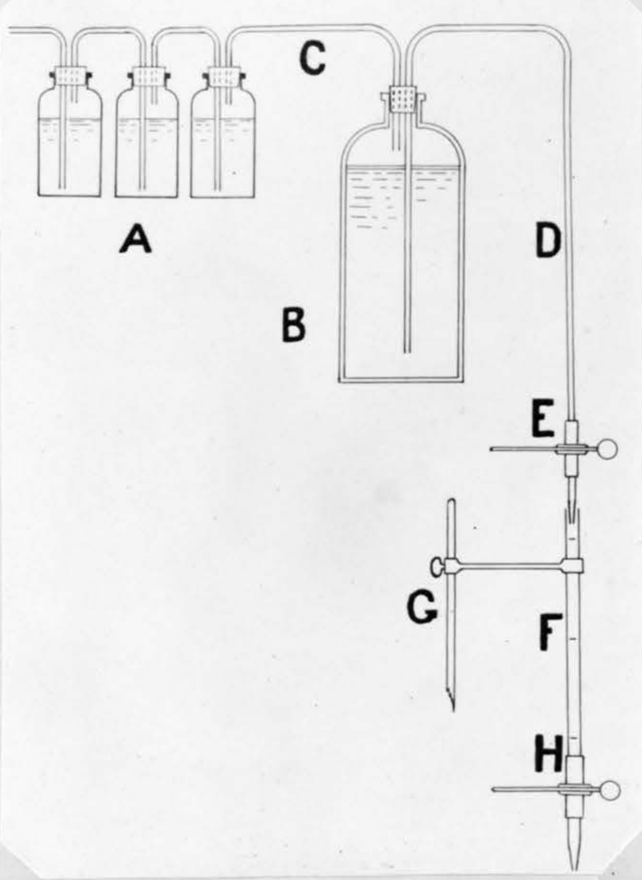

Fig. 6. Apparatus used in obtaining CO_2 free Barium hydroxide solution.

A. Wash bottles containing sodium hydroxide solution.
B. Storage vessel containing a saturated solution of Barium hydroxide.
C. and D. Glass tubing
E. and H. Spring clamps
F. Calibrated glass tube
G. Ring stand support

siphon (D) provided with a stopcock (E). A glass tube calibrated at 15 cc. intervals (F) is closed by a stopcock (H) and supported by a ring stand (G).

Five cc. of the barium hydroxide solution is drawn into the shell vial from the storage vessel by means of the siphon. The air entering the bottle to replace the $Ba(OH)_2$ being drawn out is free of CO_2 by virtue of the fact that it is drawn through the wash bottles containing the NaOH solution. The shell vial is quickly closed with a No. 7 stopper not equipped with the seed cup apparatus just described.

A measured weight of seeds which have previously been soaked and sterilized is then placed in the seed cup and quickly transferred to the shell vials by substituting the stoppers bearing the cups for the stoppers closing the vials. The numbered respirometers are then placed in a germination chamber at 20°C. for the desired period of time. At the end of each 48-hour period the stopper bearing the seed cup is quickly transferred to another shell vial with a fresh supply of $Ba(OH)_2$. The shell first used is stoppered and subsequent titration carried on at the worker's convenience.

All parts of the respirometer were thoroughly sterilized before each respiration Test. The shell vials were sterilized by dry heat in the customary method and the seed cups and stoppers were immersed in a 50 per cent alcoholic solution of mercuric chloride, strength 2:1000 for 2 to 3 minutes, and then washed with sterile water blanks.

The amount of oxygen present in a single shell is sufficient to support respiration for the six day period as evidenced by Jost (26) who states that the respiratory process is not affected by the oxygen content of the air until it falls below 2 per cent. Likewise Gurjar (18) has shown that 1 cc. of N/4 $Ba(OH)_2$ is equivalent to approximately 6 mg. of CO_2. The saturated solution used in these experiments is about N/2.5, hence the amount used, 5 cc., will absorb about 47 mg. of CO_2 and leave a safe excess of barium hydroxide for the measurement of CO_2 production for periods up to six days.

The titration method used in this work was adapted from the work of Harter and Weimer (24). The indicators used were thymol-sulphonthalein and tetra-brom-phenol-sulphontahlein. The barium hydroxide solution was not removed from the shell vial during the process of titration.

After the excess barium hydroxide is neutralized by the addition of N/4 HCl using thymol blue as an indicator, the precipitated barium carbonate is dissolved by the addition of a measured excess of N/10 HCl. This solution is then titrated against N/10 NaOH with brom-phenol blue as an indicator. The amount of NaOH needed to bring the solution to the neutral point subtracted from the amount of HCl used gives the amount of acid needed to dissolve the precipitate of barium carbonate. The number of cc. of HCl required to dissolve this precipitate may then be translated into terms of mg. of CO_2 by multiplying cubic centimeters of HCl by the factor 2.2. From the equation given below it follows that 1 cc. of N/10 HCl is equivalent to 2.2 mg. of CO_2.

$$2\ HCl + Ba\ (CO_3) = BaCl_2 + H_2O + CO_2$$

$$2000\ cc.\ N/HCl = 44\ g.\ CO_2$$

$$1\ cc.\ N/1\ HCl = .022\ g.\ CO_2 = 22\ mg.\ CO_2$$

$$1\ cc.\ N/10\ HCl = 2.2\ mg.\ CO_2$$

Relation of the method of seed sterilization and presoak period to evolution of CO_2.- Preliminary experimentation on the relation of certain methods used in these respiration studies to the output of CO_2 was necessary before the experiment proper could be undertaken. The strength of the sterilizing solution and the length of time of sterilization as well as the length of presoak period were all considered.

Calcium hypochlorite as recommended by Wilson (45) was first used. This proved decidedly ineffective in suppressing the growth of microorganisms so its use was discontinued.

Mercuric chloride was finally used to sterilize all seeds in the manner advocated by Norton and Chen (32) which is as follows. Soak seeds from 10 to 12 hours thus rendering pathogens on seed coat more susceptible to disinfectant,at the same time preventing injury to seed by disinfectant. This period of presoaking has also been suggested by Braun (9). Shake presoaked seeds from 3 to 5 minutes in a 50 per cent alcoholic solution of mercuric chloride, strength 2:1000. Remove traces of disinfectant by washing in 93 per cent ethyl alcohol and 3 times in sterile water.

Changes were made in the length of presoak and sterilizing periods and strength of solutions used to more nearly meet the requirements of this problem. Whole seeds were sterilized with both 1:1000 and 2:1000 solutions for varying lengths of time to determine which strength of solution and which length of time was the most effective and at the same time the least detrimental to seed viability. The same study was given split seeds, this type was chosen for the preliminary experimentation as being the most severely injured type of broken seed. After sterilization the seeds were plated on nutrient agar and were examined at the end of 3 and 6 days from two standpoints, fungus growth and seed growth.

The effect of varying lengths of presoak periods on susceptibility of the seeds to fungi was studied in relation to sterilization methods. The results of these experiments are given in Table 1.

As shown by this table neither the 1:1000 nor 2:1000 sterilizing solution for a period of 30 seconds or 1 minute was effective in suppressing fungal activity on whole seeds. The longer length of sterilization period, 4 and 5 minutes, was effective in keeping the infection down but had a deleterious effect upon germination. In nearly every case the mercuric chloride solution, strength 2:1000 was more effective than 1:1000 with the whole seeds. It is interesting to note the marked correlation between the length of presoak period and the efficiency of the sterilizing solution. Evidently with the whole seeds 4, 8 or 12 hours presoaking was not sufficient to render the fungi sufficiently susceptible to the mercuric chloride to kill them. Until the 24 hour presoak period bacterial infection is not evident, however, when the period for presoaking is lengthened to 24 and 48 hours, bacterial infection increases at the same time fungal infection decreases.

The germinability of split seeds is greatly decreased by the 2:1000 solution when used for lengths of time

Table 1. Effect of sterilization on infection and germination of injured wheat seed.

		Sterilization period	30 seconds		1 minute		2 minutes		3 minutes		4 minutes		5 minutes	
Presoak period	Type of seed	Strength $HgCl_2$	1:1000	2:1000	1:1000	2:1000	1:1000	2:1000	1:1000	2:1000	1:1000	2:1000	1:1000	2:1000
4 hours	Whole	Germ. %	100	100	100	100	100	100	100	80	100	90	90	70
		Infection %	100F	100F	80F	70F	70F	70F	80F	70F	70F	60F	50F	40F
	Split	Germ. %	40R	60R	60	40R	40R	0	40R	0	30	20R	20R	0
		Infection %	Free	Free	Free	Free	Free	Free	Free	Free	Free	Free	Free	Free
8 hours	Whole	Germ. %	100	100	100	100	100	100	100	100	100	80	100	80
		Infection %	90F	70F	80F	60F	60F	60F	70F	30F	40F	40F	40F	30F
	Split	Germ. %	100	20R	100	40	60R	20	80R	20R	30R	30R	40R	0
		Infection %	Free	90B	Free	Free	Free	20B	Free	Free	10B	Free	Free	10B
12 hours	Whole	Germ. %	100	100	100	100	100	100	100	100	80	70	40	40
		Infection %	90F	70F	90F	80F	80F	60F	60F	30F	70F	60F	50F	20F
	Split	Germ. %	60R	40R	30	40	40	40	20	0	20	0	0	0
		Infection %	Free	Free	Free	Free	Free	Free	20F	Free	Free	Free	Free	Free
24 hours	Whole	Germ. %	100	100	100	100	100	100	100	100	100	90R	90R	40R
		Infection %	10B 30F	10B 20F	30F	20F	10F	Free	1B 1F	Free	Free	Free	Free	Free
	Split	Germ. %	-	-	-	-	-	-	-	-	-	-	-	-
		Infection %	-	-	-	-	-	-	-	-	-	-	-	-
48 hours	Whole	Germ. %	100	100	100	100	100	100	100	100	90	90R	90	90R
		Infection %	30B 70F	20F	50B 30F	20B	20B 10F	30B 20F	10F	10B 20F	20B	Free	Free	Free
	Split	Germ. %	-	-	-	-	-	-	-	-	-	-	-	-
		Infection %	-	-	-	-	-	-	-	-	-	-	-	-

R - Growth retarded F - Fungal infection B - Bacterial infection for one minute or more. The three, four and five minute sterilization periods are quite as injurious when 1:1000 solution is used. Very little infection is found in any of the split seeds, however the germination is poor. With these seeds bacterial infection evidences itself after only an eight hour presoak period. The sterilization optimum with broken seeds is at a much lower point than it is with whole seeds as far as strength of solution, presoak and sterilization periods are concerned. Growth is also retarded in split seeds by much shorter sterilization periods than affect whole seeds.

We may conclude from this table that the optimum sterilization period for broken seeds is one minute, while that of whole seeds is two minutes. Mercuric chloride solution , strength 1:1000 is very effective for broken seeds while the higher concentration 2:1000 is required for complete sterilization of whole seeds. The most effective presoak period proved to be 8 hours for broken seeds and 24 hours for whole seeds. In accordance with these results all seed sterilization in the respiration tests was carried on in this way.

<u>Relation of length of presoak period to evolution of CO_2</u> - Several tests were made with whole seeds to deter-

mine the correlation of varying lengths of presoak periods with the rate of gaseous exchange. During the period of presoaking all seeds were kept in water at a constant temperature of $20^{\circ}C$. The results of this experiment are given in Table 2.

Table 2. The relation between presoak period and respiratory activity.

Sample Number	Presoak period 8 hrs.	Presoak period 12 hrs.	Presoak period 24 hrs.	Presoak period 48 hrs.
14A	34.18			
14B	22.74			
14C	23.62			
24A	29.86			
24B	34.11			
24C	33.31			
Average	29.63			
15A		36.00		
15B		29.80		
15C		27.17		
23A		31.61		
23B		30.73		
23C		22.00		
Average		29.55		
16A			32.43	
16B			28.92	
16C			28.10	
22A			28.92	
22B			25.52	
22C			26.35	
Average			28.37	
17A				21.91
17B				22.00
17C				21.82
21A				27.12
21B				29.74
21C				33.32
Average				25.98

It is obvious that very little difference in CO_2 production exists between the first three periods, 8 hours, 12 hours, and 24 hours, while the 48 hour period exhibits a decrease. The logical conclusion which may be drawn from these tests is that the difference in CO_2 production of seeds soaked for 8, 12, 24, and 48 hours is in inverse proportion to the amount of CO_2 they have already given off while soaking in the water to the total respiratory output.

Respiration of Broken Seed - Experimental work on CO_2 production during the respiratory process was conducted with broken and normal seed by the above described methods. It was thought that such experiments might aid in determining whether decreased germination in wheat is due solely to mechanical injury or whether such germination might be due wholly or in part to exhaustion of stored material or to decreased vitality as a result of increased respiration from breaks in the seed coats.

Using lots of wheat from various sources respiration tests were made of each of the four types of broken seeds as shown in Figs. 1 to 4 . These tests included both hand broken and machine broken seeds from the same lot with a test of the whole seed for a check. During the course of the experiment 125 respiration tests were made using the various types of wheat chosen from lots of different ages.

In the following table (3) ten lots of seeds are tabulated with their respiratory activity expressed in terms of CO_2 evolution. For purposes of comparison the lots of wheat treated in this table are the same as those discussed in the work on the effect of mechanical injury on germination. These ten lots are representative of the entire number of samples studied.

Before placing the seeds in bottles for presoaking they were weighed with a chainomatic balance, approximately 0.5 g. was used for each test. After the respiration test the amount of CO_2 evolved was calculated for each sample on the basis of one gram of seed. The respirometers were kept in a dark germinator at a constant temperature of 20°C. for 144 hours, the CO_2 evolved being measured at the end of each 48 hour interval as shown in Table 4.

In the following table (3) and accompanying graph (Fig. 7) is shown the total respiration of slightly injured, severely injured, three-fourths and split seeds, together with that of normal seed. It may be noted in these data that all injured seed respires greater amounts of CO_2 than does the uninjured seed. The three-fourths seed respires the greatest amount, 51.30 and 47.05 mg. of CO_2 for machine

Table 3. Total amounts of CO_2 respired in six-day periods.

	Machine broken seed				Hand broken seed				Unbroken
Sample Number	Slight	Severe	Three-fourths	Split	Slight	Severe	Three-fourths	Split	seed
17959	45.54	37.84	61.44	50.63	47.14	36.67	47.82	51.36	40.48
18445	46.04	52.01	49.56	47.26	39.63	36.80	45.02	39.62	34.11
18469	50.57	42.61	61.12	55.87	48.43	28.77	56.86	57.67	32.04
21118	48.41	-	-	64.70	48.50	46.36	51.56	55.91	27.82
21148	43.83	46.77	72.00	48.07	36.81	40.07	53.96	48.12	26.28
21486	50.83	-	-	45.26	45.76	43.49	56.97	47.94	25.70
21535	46.88	-	-	47.61	50.32	44.00	45.80	47.82	24.30
21613	50.89	51.87	-	-	50.28	35.98	54.15	49.08	28.36
193	27.34	26.36	31.24	28.08	27.50	25.88	27.07	27.77	27.17
194	32.17	28.71	34.44	29.22	30.43	27.77	31.24	31.18	24.83
Ave.	44.25	40.88	51.30	46.31	42.48	36.58	47.05	45.54	29.10

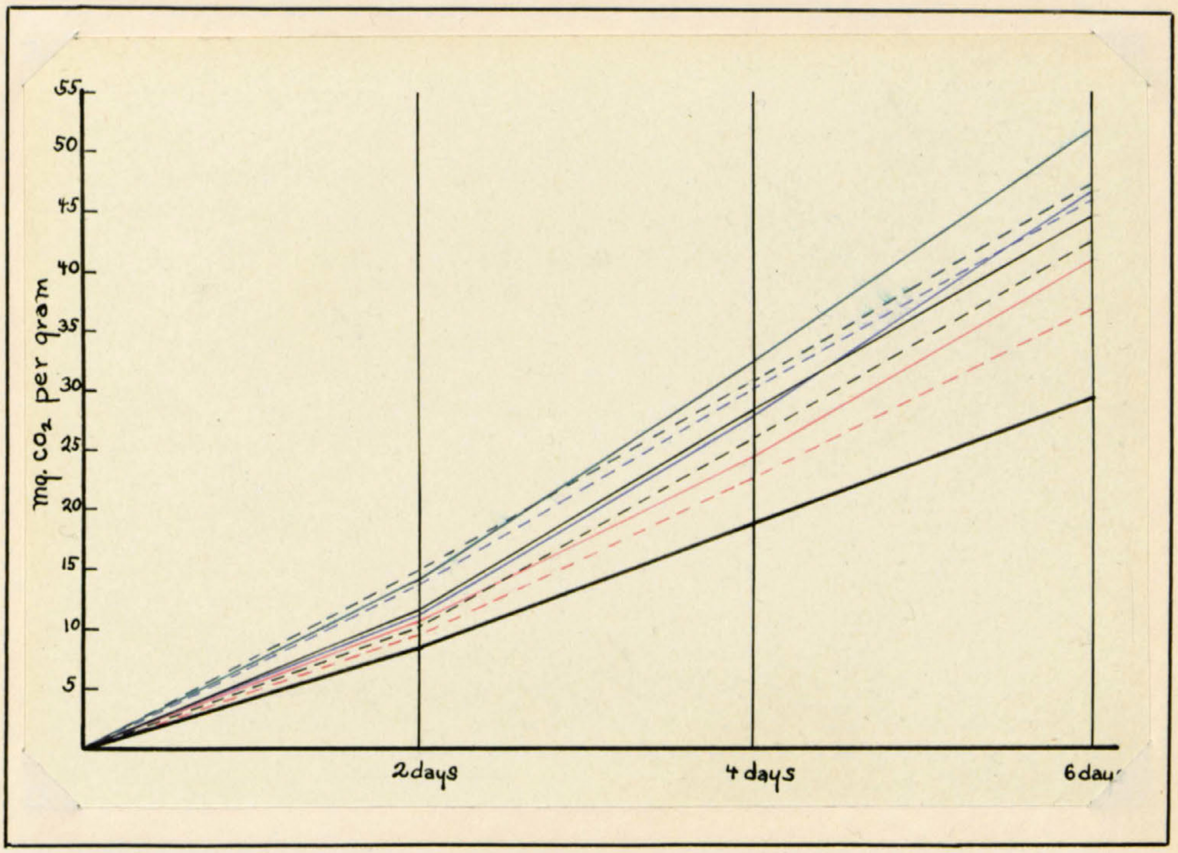

Fig. 7. Graph showing total CO_2 production over a period of 6 days expressed in mg. of CO_2

——— Machine broken, slight
- - - - - - Hand broken, slight
——— Machine broken, severe
- - - - - - Hand broken, severe
——— Machine broken, three-fourths
- - - - - - Hand broken, three-fourths
——— Machine broken, split
- - - - - - Hand broken, split
——— Check, whole seed

broken and hand broken respectively. The least CO_2 is given off by those seeds severely injured at the embryo end, 40.88 mg. for machine broken and 36.58 mg. for hand broken seeds. Since injury stimulates respiration it might seem that the split seed, being the most severely injured type, would give evidence of the greatest respiratory activity in place of the three-fourths seed. A possible explanation of this seeming discrepancy may be taken from the work of Bailey and Gurjar (7) on the relation of plumpness of wheat kernels to the rate of respiration. Basing their conclusions upon the assumption that the embryo is the respiratory and enzymatic center of the seed they find that in the shriveled grain the enzymatic activity is approximately twice as great as in normal seed. They attribute this result to the fact that the substrate or endosperm is decreased nearly half while the embryo, the source of enzymes, remains practically normal. Such an explanation can be accepted with modifications. In Part II. of this paper the theory of enzyme secretion wholly by the embryo as stated by the above workers (7) is contested; however, it is shown that weight for weight the embryo is richer in enzymes than is the endosperm. With this consideration in mind it may be possible that the three-fourths seed having lost a part of its endosperm is subjected to a wound stimulus which more than counterbalances the loss of respirable material from the broken endosperm. Since no part of the embryo is broken away the total respiratory action of a three-fourths seed is the same as that of a whole seed with the factor of injury stimulus in addition. The severely injured seed which displays the lowest germination rate loses a part of the embryo, that portion in which the enzymes are most highly concentrated. This loss is not counterbalanced by the injury stimulus presented by the small break hence respiration proceeds at a lower rate. This explanation can be applied in the same way to total amounts of CO_2 respired by the split seeds and those having a slight injury at the embryo end.

From the standpoint of respiratory activity it seems logical to assume that a freshly broken seed might behave differently from a seed in which the break has existed for some time. Therefore with every lot of machine broken seeds a parallel test was run with seeds which were hand broken at the time of testing. A comparison of these two groups is also shown in Table 3.

Normally the life of wheat seed may extend over a period of 15 years or more. Upon wounding there is an increase in the rate of respiration in stored seeds, likewise in germinating seed as shown here, and therefore it may be reasonable as stated previously in this paper, that if the speed of the respiratory process is increased it cannot last as long as it might under normal conditions. If the respiration process is a concomitant of viability, it may then logically be assumed that broken seeds will retain their vitality for a shorter length of time than will whole seeds.

A theoretical curve based upon respiration in broken seeds can be constructed with the results presented in Table 3. This table shows that the machine broken seeds respire more than the hand broken and that, in turn, both of these types respire more than do the whole seeds. It is thus possible that a curve of respiration starting at the time the seeds are broken will increase very gradually until the maximum point of CO_2 production is reached, at approximately four or five years, after which it will decrease sharply, falling below the mean CO_2 production of whole seeds considerably short of the 15-year period. Upon such a curve, CO_2 production of seeds broken for two to three years will be slightly greater than that of seeds which are freshly broken;while in whole seed respiration will proceed at a uniform rate considerably below that of broken seeds. The fact that broken seeds of all types respire more than do whole seeds indicates that loss of vitality due to a wound stimulus on respiration cannot be responsible for lowered germinability evidenced by broken seeds. A curve of respiration as described above would indicate that the rate of respiration is not an index to the vitality of broken seeds until the latter part of their longevity period.

The consideration of the rate of respiration by two-day periods in whole and in injured seed is also of interest. Readings of the amount of CO_2 evolved were made on the germinating seed at 48 hour intervals, the records of which are shown in Table 4.

In Table 4 and the accompanying graph (Fig. 8) it is shown that the evolution of CO_2 in every case whether seed is injured by hand or by machine is much greater from the first to the second period than it is during the latter two periods. During the first period there is a definite increase in CO_2 production no matter what the type of seed may be. From the second to the third period the intensity of respiration varies with the different types of breaks. In two cases machine broken slight, and hand broken

Table 4. Carbon dioxide production in milligrams from normal and injured wheat seed, readings at two-day intervals.

Sample Number	Machine broken seed												Hand broken seed												Unbroken seed		
	Slight injury			Severe injury			Three-fourths			Split			Slight injury			Severe injury			Three-fourths			Split					
	2 das.	4 das.	6 das.	2 das.	4 das.	6 das.	2 das.	4 das.	6 das.	2 das.	4 das.	6 das.	2 das.	4 das.	6 das.	2 das.	4 das.	6 das.	2 das.	4 das.	6 das.	2 das.	4 das.	6 das.	2 das.	4 das.	6 das.
17959	8.59	17.19	19.76	7.92	14.08	15.84	18.33	22.56	20.54	12.22	18.33	20.08	10.48	17.46	19.21	7.67	11.09	17.91	16.52	13.91	17.39	13.70	17.12	20.54	9.68	12.32	18.48
18445	13.64	16.20	16.20	14.50	17.05	20.46	16.52	15.65	17.39	11.17	15.47	20.62	9.81	10.18	19.63	10.27	11.98	14.55	14.44	17.84	12.74	14.60	16.39	8.63	7.00	13.56	13.56
18469	14.33	18.54	17.70	8.69	14.78	19.13	14.65	19.91	26.55	13.97	18.33	23.57	11.49	15.60	21.34	10.15	6.77	11.85	16.78	17.71	22.37	15.42	18.50	23.64	9.09	10.83	12.12
21118	3.59	17.84	16.99	-	-	-	-	-	-	12.94	26.96	24.80	9.53	19.05	19.92	8.43	17.70	20.23	16.33	17.19	18.05	14.91	20.00	21.00	8.56	10.27	8.99
21148	11.58	17.37	14.89	12.12	13.86	20.79	16.00	29.33	26.67	11.41	17.92	18.74	6.85	17.98	11.98	9.38	15.35	15.35	16.60	18.26	19.09	16.33	15.47	16.33	7.63	8.48	10.17
21486	11.91	19.06	19.85	-	-	-	-	-	-	12.13	15.37	17.79	10.44	17.66	17.66	11.08	16.20	16.20	15.78	20.16	21.03	13.70	20.54	13.70	9.58	7.84	8.28
21535	11.71	20.91	14.26	-	-	-	-	-	-	13.13	19.70	14.77	10.32	21.44	18.55	12.00	17.60	14.40	14.72	16.36	14.72	15.65	17.39	14.78	7.67	8.53	8.10
21613	13.95	18.88	18.06	15.20	18.78	17.89	-	-	-	-	-	-	14.14	18.86	17.28	10.63	13.08	12.27	16.92	18.61	18.61	12.14	20.15	16.79	8.59	9.45	10.31
193	6.83	9.82	10.68	6.91	8.64	10.80	8.48	11.60	11.16	6.80	8.94	12.34	7.50	10.00	10.00	8.35	8.77	8.77	8.46	9.73	8.88	9.20	8.36	9.20	7.33	10.35	9.49
194	8.69	12.17	11.30	7.60	10.13	10.98	9.68	11.00	13.76	7.30	11.17	10.74	9.13	11.30	10.00	7.26	10.68	9.82	7.70	12.84	10.70	10.39	10.39	10.39	7.84	11.33	5.66
Ave.	11.48	16.80	15.97	10.42	13.90	16.56	13.94	18.34	19.34	11.23	16.91	18.16	9.97	15.95	16.56	9.52	12.92	14.13	14.42	16.26	16.36	13.60	16.43	15.50	8.30	10.30	10.52

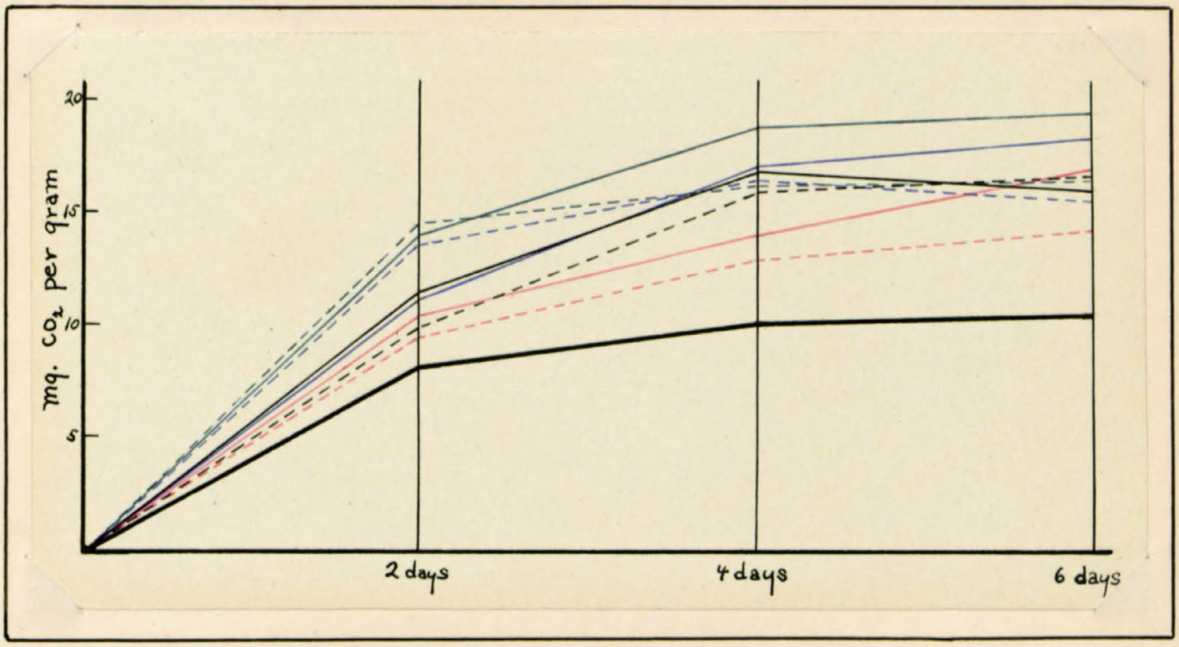

Fig. 8. Graph showing CO_2 production from wheat seed for two-day periods, expressed in mg. of CO2

——— Machine broken, slight
------ Hand broken, slight
——— Machine broken, severe
------ Hand broken, severe
——— Machine broken, three-fourths
------ Hand broken, three-fourths
——— Machine broken, split
------ Hand broken, split
——— Check, whole seed

split, the amount of CO_2 given off during the third period falls below the amount given off during the second period. In every other case the rate of evolution during the third period exceeds the rate during the second period but slightly. We may conclude from this that the maximum intensity point of respiration is reached during six day's time in two samples and is closely approached in the others. It can also readily be seen that the intensity of CO_2 production by the machine broken seeds, period for period, is not quite reached by the hand broken seeds.

The Mechanical Effect of Injury on Germination of Wheat

The injury to wheat seed received in threshing may not only be reflected in changed rate or degree of respiration but in germination. The most obvious effect of breakage in fact is the influence on the character of germination. The breaking away of part of the embryo may result in abnormal shoot and root development or the injury to the seed may deprive the embryo of food or expose the tissues of the embryo to excess oxidation.

Regarding the first type of injury of the seed Brown (11) concludes, from a series of experiments with mutilated germs of _Zea mays_ that the entire embryo is not essential to germination and development into normal plants. Contrary to this work Lute (29) finds that all breaks in the seed coats of wheat, rye, barley and oats are deleterious to germination, injury to the proximal end being more serious than injury to other parts of the seed. This conclusions is demonstrated both by per cent of germination and total growth produced.

The second type of mechanical injury may produce its effect upon germination by decreasing the food supply of the growing embryo through the loss of a part of the endosperm. Such effect is characterized by a general retardation of growth rather than an acutal abnormality in growth as produced by an injured embryo.

Andronescu (1) shows that excised embryos of *Zea mays* will produce plants in which growth is generally retarded but whose root systems are unimpaired. Working upon the effect of endosperm injury to the growth of *Zea mays*, Brown (11) has found that stands from mutilated seed were lower than those from whole seeds, he also finds that the growth of such plants as did develop was retarded. The results obtained by Brenchley (10) from comparative experiments with light and heavy seeds and their effect on the resulting crop, lend support to the practice of sowing large heavy seed. Schmidt (38) reports that when plants are grown under the same conditions the superiority of those grown from heavy seed over those grown from lighter seeds decreased greatly as the plants approached maturity, sometimes disappearing entirely. To a certain extent we can apply these results to the problem in hand insofar as the amount of food material furnished by the remaining endosperm in a broken seed is concerned.

The lack of agreement in the literature on this subject and such observations as have been made by the writer would indicate that the factor of decreased food supply is not of primary importance. It is probable that under favorable soil and weather conditions, with the only adverse factor being the amount of available food material, the difference in total growth produced by a whole seed and one in which a small part of the endosperm is broken away is negligible.

Germination of Broken Seeds.— As a means of determining the possible effect of mechanical injury, in a purely physical sense, upon the subsequent germinating capacity of wheat, a series of germination tests was made, the results of which are recorded in Table 5. Controlled temperature conditions were used in all of these tests. The seeds were placed in a germinating chamber at 20°C., the optimum recommended by the American Association of Official Seed Analysts (44). Preliminary germination counts were made at the end of three days, and the final count was made on the sixth day. In the tests recorded in Table 5 only those seeds were considered as germinated which had put out a plumule, primary root and the first pair of lateral roots as illustrated in Fig. 9, taken from Percival (35). Figures 10 to 14 show typical germination of wheat exhibiting the four types of breaks taken at the end of the fourth day in the germinator. The uninjured seed (Fig. 10) shows perfect development. The

Table 5. The effect of different types of breaking on the germination of wheat seed.

	Germination Percent								
	Machine broken seed					Hand broken seed			
Sample Number	Whole check	Slight injury	Severe injury	Three-fourths	Split	Slight injury	Severe injury	Three-fourths	Split
17959	99	40	20	80	60	100	60	100	80
18095	100	80	80	80	60	100	0	100	100
18100	100	80	100	100	20	100	100	100	40
18445	99	100	20	100	40	100	40	100	80
18469	98	60	60	80	60	100	40	100	60
21118	100	100	-	-	60	100	60	100	100
21148	99	100	-	-	100	100	40	100	40
21486	100	100	-	100	40	100	40	100	60
21535	100	100	-	-	60	80	20	80	80
21613	100	100	-	-	-	100	100	100	20
21617	100	100	-	90	-	100	100	100	100
21719	99	60	80	-	0	100	0	100	40
21907	100	60	-	-	80	80	60	100	80
21934	99	60	80	-	80	100	80	100	80
Ave.	99.5	81	63	90	55	97	62	99	69

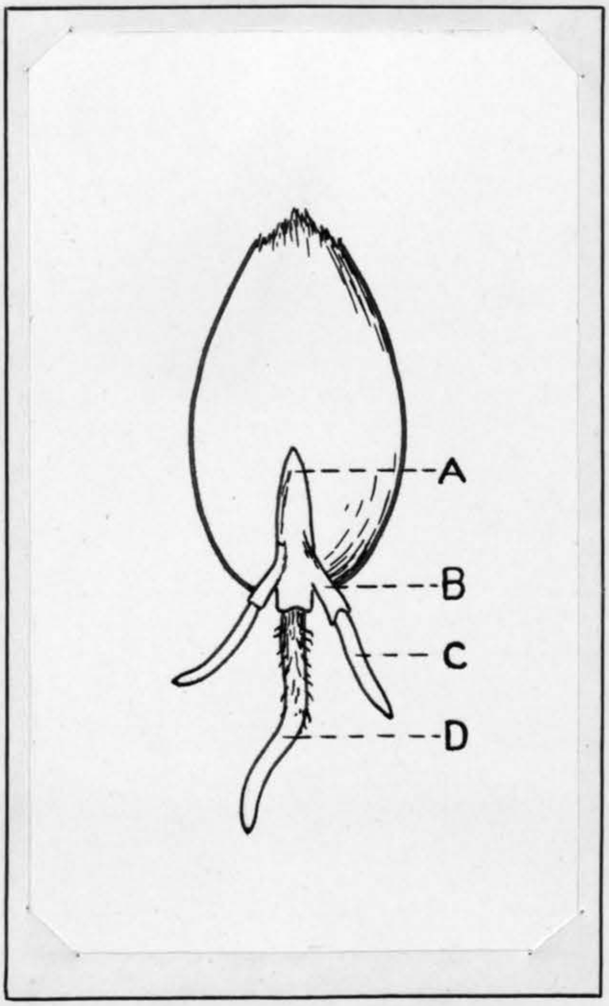

Fig. 9. Normal germination of
wheat seed
A. Plumule
B. Coleorhiza
C. One of first pair of
lateral roots
D. Primary root
(From Percival)

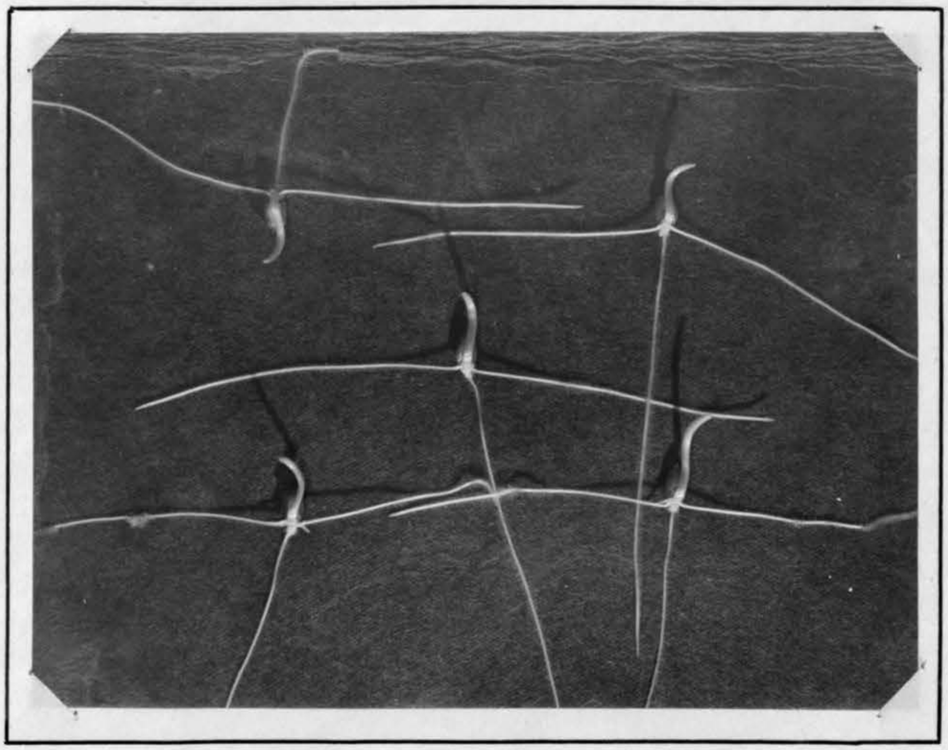

Fig. 10. Typical germination of whole wheat seeds.

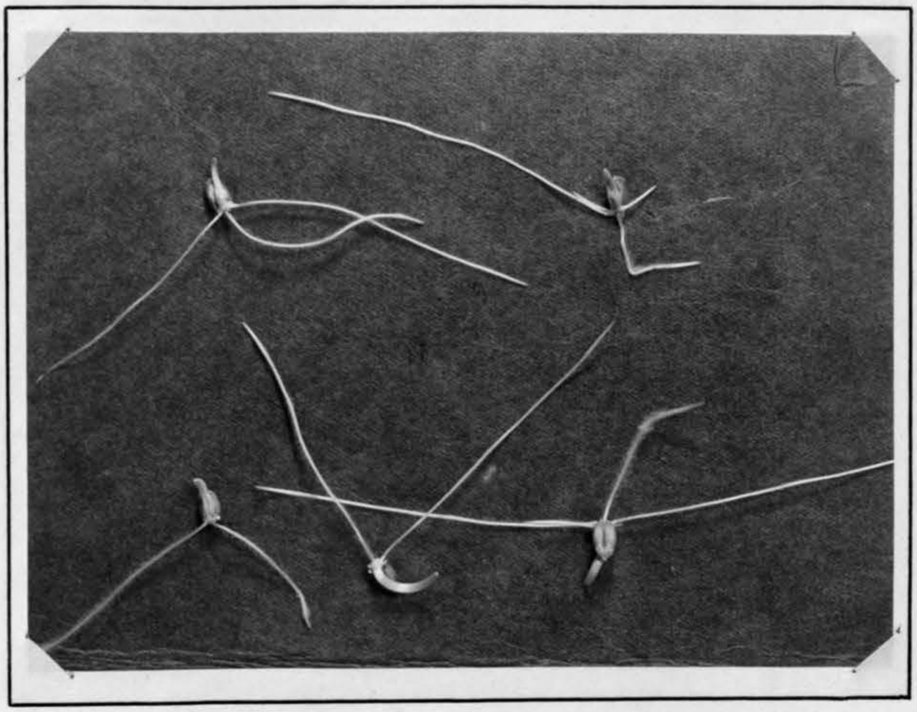

Fig. 11. Typical germination of wheat seeds with slight injury at embryo end.

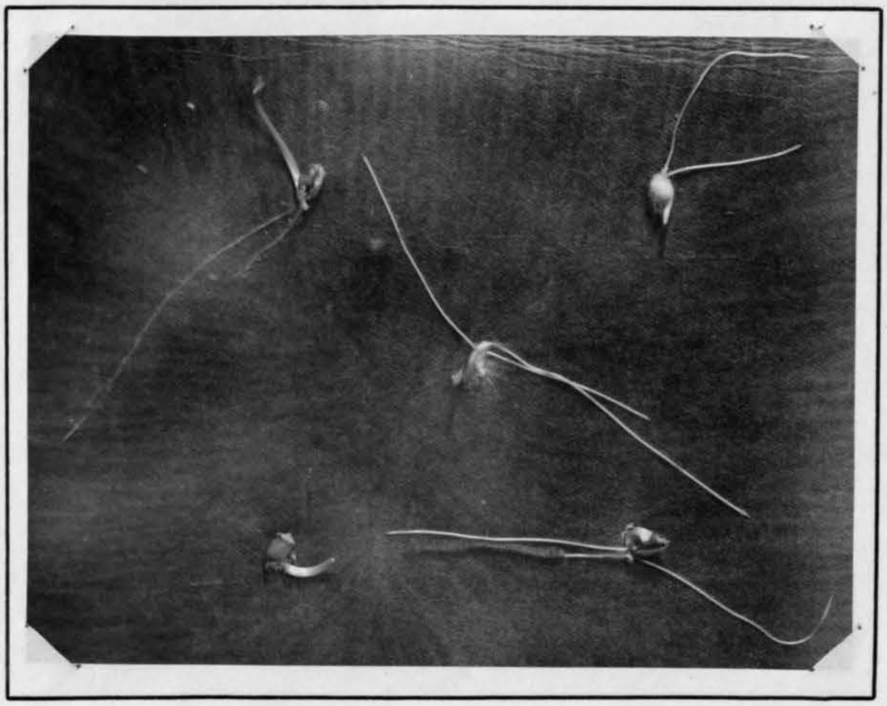

Fig. 12. Typical germination of wheat seeds with severe injury at the embryo end

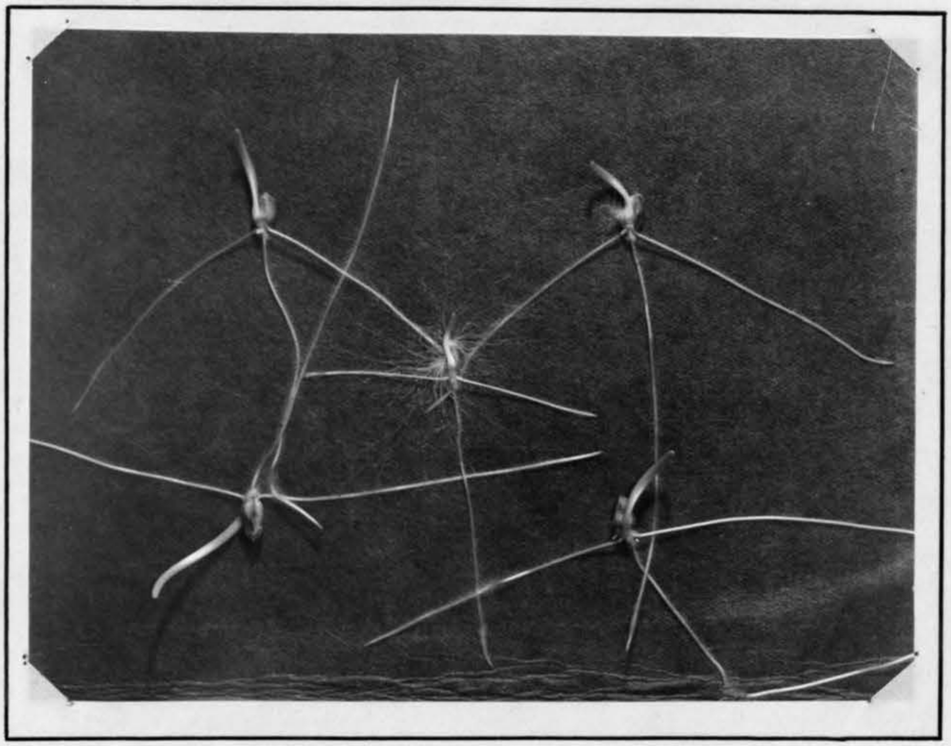

Fig. 13. Typical germination of wheat seeds with three-fourths injury, at the brush end

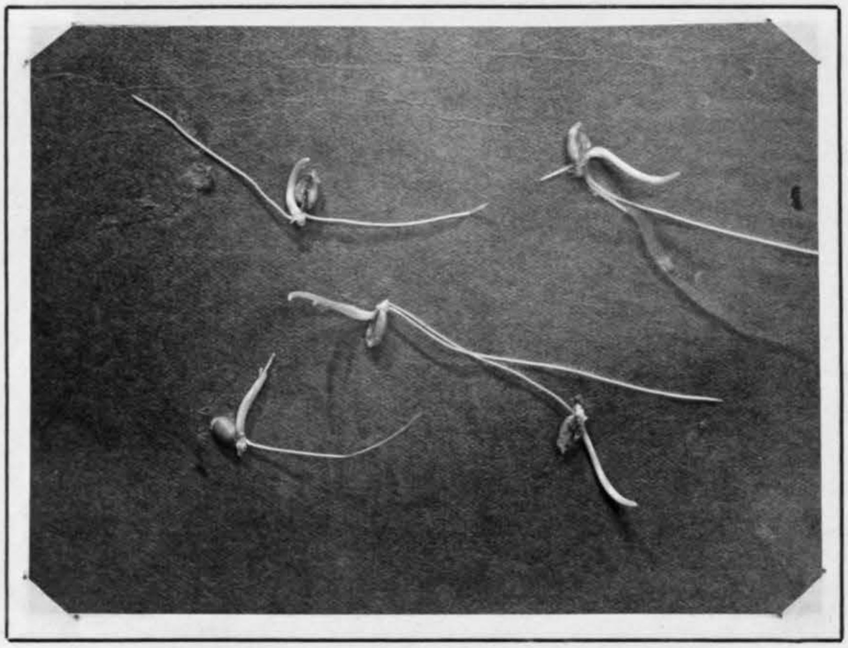

Fig. 14. Typical germination of wheat seeds , split lengthwise.

development of the seed slightly injured at the embryo end (Fig. 11) is slightly retarded with imperfect root development and plumule sheaths which show signs of splitting. The germination of severely injured seed (Fig. 12) is very poor showing lack of root and shoot development in some cases. Fungal activity is also evident. The germination of the three-fourths seed (Fig. 13) is practically normal with the exception of fungal infection. Split seeds (Fig. 14) are characterized by retardation of growth with lack of root development in some cases. Two of the plumule sheaths are split indicating a poor stand under field conditions.

A consideration of the above mentioned table (5) and Figures 10 to 14 shows that mechanical injury does have a deleterious effect upon germination. Further, lowered germination is proportional to the type of break incurred by the seed. The three-fourths seed and slightly injured seed display the least visible effect of injury while germination is considerably retarded by severe injury to the embryo. The germination of split seeds is only slightly over half that of the whole seeds. Seeds which are hand broken give more favorable results than those with older breaks received during the threshing process. The three-fourths and slightly injured seeds thus broken germinated 99 per cent and 97 per cent respectively which compares favorably with whole seed germination, 99.5 per cent.

The serious nature of the break in the severely injured seed doubtless accounts for the fact that the germination of hand broken seeds of this type is approximately the same as the machine broken. This is due to the broken and mutilated state of the embryo causing equally poor germination in both old and newly broken seed. It is obvious that the broken seed in which the embryo is badly injured gives the lowest germination for in seeds where the embryo end is severely injured and in split seeds the germination falls as low as 62 per cent for the first type and 69 per cent for the second. This would seem to indicate that low germination results from injury to the embryo. Such behavior is quite reasonable and indicates the direct and harmful effect of mechanical injury upon germination.

The suggestion has been made that lowered germinability of broken seeds may simply be the result of a decreased supply of food material for the young plant caused by breaking away part of the endosperm. As a matter of fact the three-fourths seed gives a higher germination than any of the other broken types which belies this supposition.

From the data just reviewed it is apparent that mechanical injury does cause lowered germination in wheat seed and that the percentage of germination is decreased markedly more by injury to the embryo or proximal end than by injury to the opposite or distal end of the seed. The fact that hand broken seeds display a slightly higher germination than do the seeds with breaks received at the time of threshing evidences the effect of deleterious factors other than injury which is purely mechanical by which germination may be reduced. Chief of these factors may be a loss in viability due to intensified respiration rate during storage.

Conclusions

Wheat seeds broken by threshing are always found in varying proportions in samples received for test at the state seed laboratory. The disposal of those pieces which are larger than half a seed presents a problem to the seed analyst since the germinability of the lot of seed as a whole is lowered by the broken ones.

Of four different types of breaks which occur most commonly in wheat, slight injury at the embryo end, severe injury at the embryo end, three-fourths seeds with the injury at the brush end and split seeds, the slightly injured and split seeds make up approximately 90 per cent of the broken seeds.

The respiration of wheat seeds is increased markedly by breaking, the increase in respiration varying with the type of break. The three-fourths seeds evidence the greatest respiratory activity, followed by split, slightly injured and severely injured seeds; unbroken seeds respire at a much lower rate than any of the broken seeds.

The fact that seeds which are hand broken at the time of testing respire less rapidly than seeds broken two to three years previously indicates the presence of a longevity factor which may cause a variance in respiration rates during the life of the seed. The relation of such a factor to the rate of respiration may be illustrated by means of a theoretical curve of broken seed respiration. Under normal storage conditions the curve of respiration increases gradually until the point of greatest respiratory intensity is reached. This may be approximately four to five years after breaking,using seeds broken shortly after maturity, the length of time depending upon the normal life of the seed. After this maximum point is attained a sharp decrease takes place reaching zero, the point where the seed is no longer viable, probably a considerable time short of the 15-year old period, approximately the normal life of wheat seed. Between the maximum point and the zero point it is possible that respiration may act as an index to vitality. If such a curve were drawn it would show the respiration of seeds broken at the time of threshing, two to three years previously, at a higher point than seeds broken at the time of testing. The curve would also show that broken seeds with consequent accelerated respiration rates would remain viable for a shorter length of time than would whole seeds in which respiration proceeds at the normal rate. The conclusion may also be reached that until the maximum point of the respiratory curve is reached the increased respiration rate due to wounding has no deleterious effect, in itself, upon seed viability. Wheat seed used for planting is generally not over two years old hence the effect of increased respiration rates on such seeds is negligible.

The rate of respiration in broken seeds during germination was found to be comparatively low for the first two days, increasing rapidly thereafter up to the sixth day after which it soon reaches the maximum. In two cases the maximum was reached before the six-day period.

The characteristic low germination of broken seeds varies with the type of breaking the seed has undergone. Injury is not so apparent in slightly injured and three-fourths seeds as it is in severely injured and split seeds, however, germination is noticeably lowered by all injuries to the embryo end.

It is obvious that decreased germination of broken wheat seeds as presented in this paper cannot be attributed to a loss of vitality through respiration, it must therefore be concluded that such lowered germination is due to the direct effect of mechanical injury on the seed.

PART II. THE OCCURRENCE AND BEHAVIOR OF EMBRYOLESS WHEAT SEEDS.

Introduction - The occurrence of wheat caryopses entirely devoid of embryos but perfectly normal as to endosperm development has not previously been reported. Harlan and Pope (20) working with barley are the only experimenters who record data concerning a similar phenomenon. They attribute this abnormality to single fertilization. Evidently, according to these workers, only the endosperm fertilization takes place in the development of the embryoless seed. Wheat seeds exhibiting this characteristic have been observed many times by the writer during the course of a study upon the respiration of seeds injured by threshing. It has been found that such embryo-lacking seeds respire and give evidence of enzyme activity though the embryo and epithelial layer are absent. This is of particular physiological interest as it offers the first opportunity of making a study of the respiration and enzyme activity of embryo-free seed without the error introduced by excising the embryos.

Occurrence of Seeds without Embryos - The rarity of this abnormal development in barley, as reported by Harlan and Pope (20) was not found by the writer to be equally apparent in wheat. These workers found only five seeds completely devoid of embryo tissue among many thousands of barley seeds examined while the writer reports the occurrence of such seeds in each lot of wheat received at the Colorado Seed Laboratory during the past year. To obtain enough material with which to conduct experiments on respiration and enzyme activity, it was necessary to examine minutely large numbers of wheat seeds. In connection with this careful scrutiny of some 150,000 wheat caryopses it was estimated that approximately 0.1 per cent of the seeds of 20 different lots of wheat had developed no embryo. The lots of wheat examined were representative of various varieties of both spring and winter wheat, specifically, Kanred, Turkey red, Kota, Defiance and Marquis. It appears from the above that the occurrence of embryo-lacking seeds in wheat is neither uncommon nor limited to one variety.

Embryo-free seeds closely resemble normal seeds but upon careful examination a deep depression may be noted at the point where embryo development normally occurs. Figures 15, 16 and 17 illustrate the appearance of the embryo-deficient seeds as compared with a normal seed. In Figure 17 it can be readily seen that no embryo development has taken place.

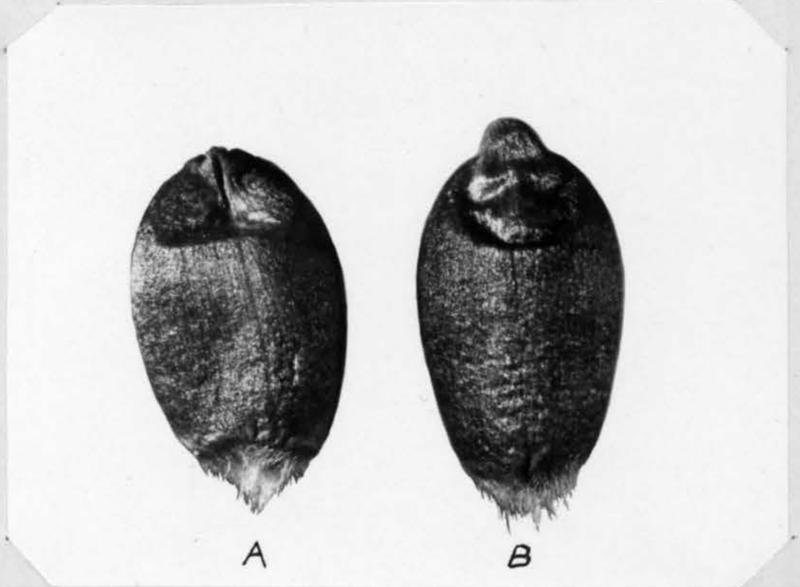

Fig. 15. Photograph of an abnormal (A) and a normal (B) wheat seed. Note the deep indentation at the proximal end of (A) where the embryo would normally be located.

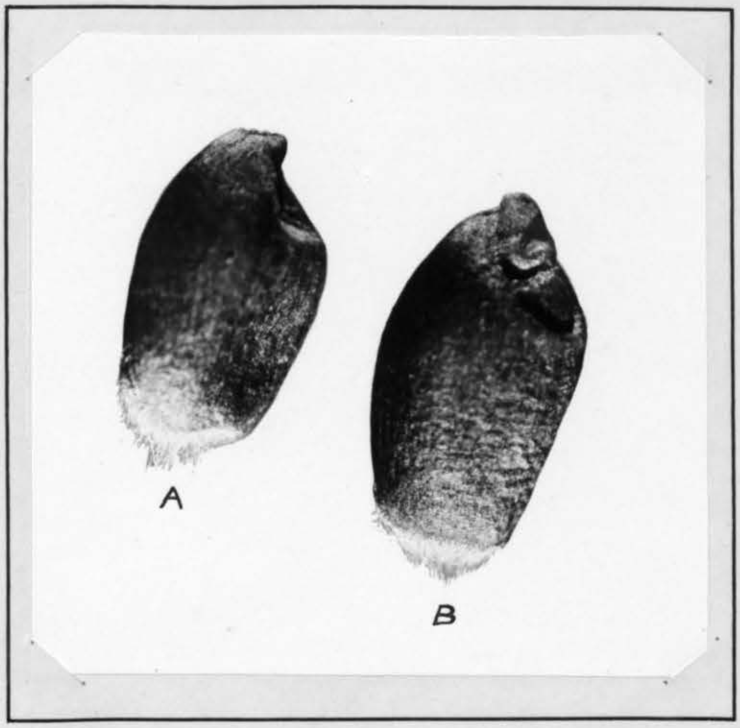

Fig. 16. Profiles of seed (A) lacking embryo and (B) normal seed.
In (A) the depression due to lack of embryo is very evident.

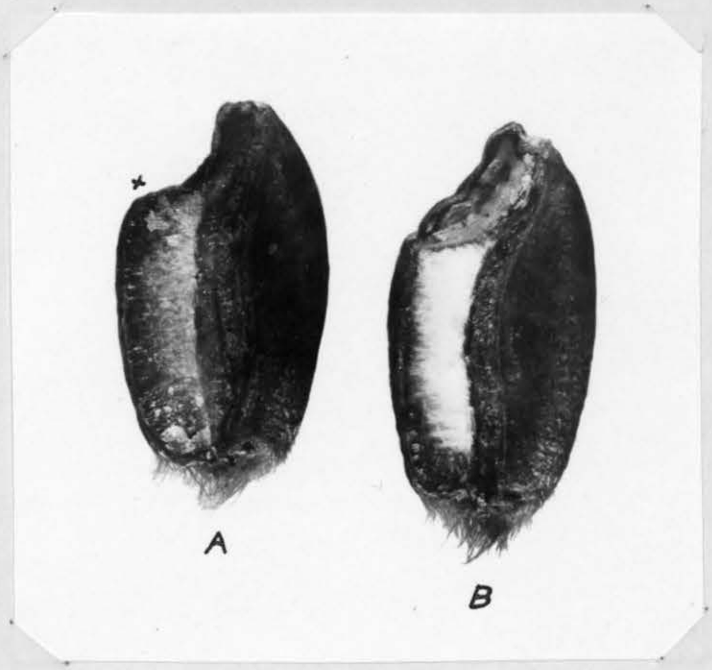

Fig. 17. Structural differences in longitudinal sections of (A) abnormal and (B) normal caryopses of wheat. All embryo tissue is lacking in (A) the embryoless seed.
The region designated X on this figure corresponds to the region designated X on Fig. 18.

Respiration of Embryoless Seeds - While it is obvious that an embryo-free seed will not grow when subjected to conditions suitable for germination the absence of the embryo in these abnormal seeds suggests the problem of determining whether or not such seeds exhibit vital processes which accompany germination of the normal seed, such as enzyme activity and respiration. Accordingly a study was made of the catalase activity and the liberation of CO_2 through the respiratory process as a means of determining the activityof these seeds which develop no embryo.

Various investigators have attributed the secretion of enzymes in the Gramineae to three different sources: first, to the scutellum, second to the aleurone layer and third to the amylaceous cells of the endosperm. The secreting power of the epithelial layer of the scutellum in greater or less degree, has been noted by practically all investigators, some going so far as to ascribe all secretion to that organ alone. Mann and Harlan (30), and Brown and Morris (12) from their physiological studies made on barley , and Torrey (43) working on germinating maize conclude that the entire source of diastase and catalase during germination is localized in the epithelial layer of the scutellum. Working with Stoner wheat, Crocker and Harrington (16) found that catalase activity of the embryo is 28 to 29 times greater than that of the endosperm. Bailey and Gurjar (7) consider the wheat embryo the seat of respiration. They also state that it is very much richer in enzymes than is the endosperm and conclude that respiration is decidedly greater in the embryo if not wholly confined to it.

From his experiments with the whole seeds and excised embryos Burlakov (14) concludes that wheat embryos respire much more vigorously, weight for weight, than do entire seeds, the respiratory ratio being 1 to 16. This conclusion is also supported by observations of Karchevski (27) who states that in the wheat embryo the rate of respiration is twelve times greater than in the entire seed. According to Kolkwitz (28) the half of a wheat seed containing the embryo respires three times as much CO_2 as the distal half when equal weights of material are compared.

Fewer investigators favor the theories of endosperm secretion which assign vital activity to either the aleurone layer or the starchy endosperm. Bruschi (13) experimenting with several species of the Gramineae maintains that the amylaceous endosperm can digest itself in varying degrees in the different seeds studied. This self-digestion is made possible because of the fact that much more pro-enzyme exists in the endosperm cells than in the scutellum and action of this enzyme may cause starch hydrolysis without aid of the embryo. Miss Bruschi's conclusions also indicate that vitality is possessed by the aleurone cells. In like manner Stoward (41) working with barley, maize, and castor beans concludes that the pure endosperm tissue of both barley and maize is capable of evolving CO_2 through the respiratory process. These investigators also attribute a part of the endosperm respiration to the aleurone layer.

All previous work as above noted, has been based on studies of seeds which contained both embryo and endosperm and in order to conduct separate respiration or enzyme tests of these two portions it was necessary to excise the embryos, thus introducing a factor of mechanical injury which might give misleading results.

It has been suggested by Stoward (41) that the greater respiratory intensity of isolated embryos may be attributed in part to a wound stimulus recieved during their removal from the seed. The work of Tashiro (42), on wheat seed stimulated by injury also bears out this statement for he shows a marked acceleration in respiration due to injury or wound stimulus. The possibility of error due to injury in separating embryos from their endosperms cannot be overlooked.

The embryoless seeds therefore present a convenient means of eliminating the effect of stimulus to CO_2 production resulting from wounding.

Sufficient embryo-free seeds were found to make possible tests of uninjured seed containing no embryo structures. To what extent respiration of the normal whole seed is due to the embryo may then be determined by comparison of such seeds with uninjured normal seeds. In order that the results of these studies might be considered comparable with the work described in the first section of this paper the apparatus and methods previously described were used in all of the respiration tests.

An average of the results obtained from respiration tests of entire normal wheat seeds shows a liberation of 26.55 mg. of CO_2 per gram of seed in six days, as compared with 22.09 mg. of CO_2 evolved by embryo-lacking seed under conditions favorable to germination. There being no embryo present this activity can only be attributed to the endosperm. From the above figures it would appear that the embryo is responsible for approximately one-sixth of the total CO_2 given off by a respiring wheat seed. It is to be remembered, however, that by weight the embryo represents but three per cent of the normal seed and yet weight for weight, respires about six times as much as the endosperm. The interesting fact, is that the experiment was conducted in a way to obviate CO_2 evolution due to injury or to microorganisms, and shows that uninjured endosperm respires and in a measure not to be inferred from previous studies.

Enzyme Activity of Embryoless Seed - It is generally believed that all respiration in plants and animals is coexistent with enzyme action. According to Bailey and Gurjar (7) that structure of the seed which is the center of respiration gives evidence of the greatest catalase activity. Crocker and Harrington (16) , Appleman (3, 4) , Morinaga (31) and Choate (15) are among those who have found a rather close correlation between respiratory action and catalase activity. Osterhout (33) states that the cessation of the respiratory process is a test for life but that its continuance cannot be accepted as a criterion, since CO_2 evolution often continues in an organism which has been killed in certain ways. The study of the respiration of embryo-free seed therefore has been accompanied by tests for the activity of amylase as well as catalase.

Small amounts of reducing sugars indicating the presence of amylase were found in the imbibed embryoless seeds after they were kept for six days under conditions suitable for germination.

In testing for catalase the simplified Bunzel apparatus was employed. Appleman's (2) methods were used in conducting the tests. The tissue was ground in a mortar with a small amount of sand and an excess of calcium carbonate. In every case, 0.025 gram of this powdered material was placed in the catalase tube with 5 cc. of the Oakland Chemical Company's commercial product, Dioxygen, which had previously been neutralized with N/10 sodium hydroxide. Readings were taken at the end of five minutes.

The results of catalase tests with whole normal seeds showed an average of 0.17 cc. of oxygen liberated during a five-minute period for every 0.025 gram of material used, whereas in the case of embryo-lacking seed 0.12 cc. of oxygen were evolved per 0.025 gram of powdered material in the same length of time. These figures indicate that as a result of the catalase activity of a wheat seed 0.12 cc. of oxygen is liberated by the endosperm while but 0.05 cc. is evolved by the embryo. This enzyme activity of the endosperm might be inferred from the respiration data given above even though all embryo structures are absent.

<u>Structure of Embryo-free Seeds</u> - It is generally believed that enzyme secretion as well as the respiratory process is

localized in the epithelial layer of the scutellum. In view of the foregoing data on respiration and enzyme activity a morphological study of seeds in which the embryo has failed to develop might be expected to show a trace of embryo tissue or at least some epithelial cells. Many sections of such abnormal seeds were made, but no indication of any embryo development was observed. Figure (18) shows a photomicrograph of a longitudinal section of an abnormal wheat seed, the lack of embryo structures in this seed is quite apparent upon comparison with a section of a normal wheat seed as illustrated by Percival (35). No trace of the epithelial layer of the scutellum is evident and all other embryo tissue is absent. The same figure illustrates the junction of the endosperm and the portion normally occupied by the distal end of the embryo. The coat structures are normal, and as in a seed with an embryo, the aleurone cells over the embryo-deficient portion of the seed are greatly diminished in size. The accompanying illustrations exhibit a marked similarity to those of barley figured by Harlan and Pope (20)

Conclusions.

Embryoless seeds are of frequent occurrence in wheat. Approximately 0.1 per cent of the lots examined manifest this abnormality. Although the endosperm is normally developed

Fig. 18. Photomicrograph of a section from an abnormal wheat seed taken in the region where the embryo would normally develop.

A. Amylaceous cells of the endosperm.

B. Collapsed cells apparently of the endosperm tissue.

C. Aleurone layer. Note the change in shape of the cells at the point where the aleurone layer of the endosperm joins with that of the embryo cavity, also that no epithelial layer is present.

The region designated X on this figure corresponds with the region designated X on Fig.17.

no trace of embryo tissue is found in micro-section.

While these seed cannot grow they do respire when placed under conditions favorable to germination and they also exhibit enzyme activity.

It has been found that wheat devoid of embryos respire 22.09 mg. of CO_2 per gram of seed in six days while normal seed give off 26.55 mg. of CO_2 in the same time. It is evident from these figures that the endosperm contributes largely to the respiration of whole normal seeds. The embryo constitutes but approximately 3 per cent of the entire seed by weight, on a weight basis therefore the respiratory activity of the embryo is decidedly greater than that of the endosperm.

Brief tests show some starch conversion in the embryo-lacking seed after six days in the germination chamber.

Further study shows a catalase activity in these seeds equivalent to 0.12 cc. oxygen production in five minutes as compared to 0.17 cc. oxygen produced in the same length of time by equal weights of normal seed.

Various investigators have studied the respiration of the embryo in its relation to the entire seed, but always after excising the embryo. The occurrence of embryo-lacking seeds offers a solution to the questioned effect of mechanical injury in studying respiration of the embryo as compared to the endosperm.

SUMMARY.

1. Seeds broken during the threshing process are of common occurrence in wheat seed.

2. The breaks of most general occurrence are:

    1. Slight injury to the embryo end
    2. Severe injury to the embryo end
    3. Three-fourths seed, injury at the brush end
    4. Split seeds.

3. The slightly injured and split seeds comprise approximately 90 per cent of all the broken seeds.

4. The optimum sterilization period for broken seeds is one minute while that for whole seeds is two minutes.

5. Mercuric chloride solution, strength 1-1000 was found to be effective for broken seed sterilization . Whole seeds required a 2-1000 solution.

6. Presoak periods of 8 hours for broken seeds and 24 hours for whole seeds proved to be most desirable for the respiration tests.

7. Breaking of wheat seeds causes a considerable increase in the rate of respiration.

8. The total amount of CO_2 produced during the six-day tests was greatest in the case of the three-fourths seed, less for the split seed, still less for the slightly injured seed and least for the severely injured seed.

9. The seeds which were hand-broken at the time of testing display lower respiration rates than those broken at the time of threshing.

10. The rate of respiration constantly increased during the six-day germination period, the curve of CO_2 production showing that the maximum intensity point was closely approached by the sixth day, in some cases and reached in others.

11. Low germination is characteristic of broken seeds.

12. Breaking away a portion of the endosperm, as in three-fourths seed causing a lessened supply of food material has no highly injurious effect upon germination.

13. The three-fourths seed displays the highest germination, followed by slightly injured, severely injured and split seeds respectively.

14. The lowered germination of broken wheat seeds as presented in this paper may be attributed, principally, to a direct effect of mechanical injury upon the embryo.

15. Embryoless seeds are of frequent occurrence in wheat, to the extent of approximately 0.1 per cent in every lot of wheat examined.

16. These embryo-free seeds respire and give evidence of enzyme activity, both catalase and amylase.

17. There is no portion of the scutellum or its epithelial layer present in these seeds, physiological activity must then be attributed to the endosperm.

18. The occurrence of embryo-lacking seeds offers a solution to the questioned effect of mechanical injury in studying respiration of the embryo as compared to the endosperm.

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