EFFECTS OF METHYL BROMIDE FUMIGATION ON THE VIABILITY OF BARLEY, CORN, MILO, OATS AND WHEAT SEEDS

by

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INTRODUCTION AND REVIEW OF LITERATURE

It is commonly recognized that fumigation of seeds with methyl bromide may cause injury to their viability. In view of the fact that methyl bromide is a highly effective fumigant, it is desirable to know how much of this gas different seeds can tolerate under different conditions without damage to viability. Several varied and scattered bits of this type of information have accumulated, including work by the State of California (1955), Cotton and Frankenfeld (1955), Cotton, et al. (1946), Fisk and Shepherd (1938), Lindgren et al. (1955), Mackie (1938), Mayer and Gammon (1957), Piper and Davidson (1938), and Walkden and Schwitzgebel (1951); however, to the knowledge of the author, no coordinated extensive tests have been conducted. Further reference will be made to the work cited above in the "Results" section of this thesis.

At the present time tests similar to those described in this paper are being conducted by Strong and Lindgren (1957) at the University of California and by King and Garner of the Entomology Department, Texas A. and M. College, College Station, Texas. Further tests with methyl bromide and other fumigants are currently in progress at the United States Department of Agriculture, Stored-Products Insect Laboratory, Manhattan, Kansas, where the tests reported herein were conducted.

The purpose of these tests was to determine the effects of different dosages of methyl bromide on the viability of five kinds of cereal seeds when treated under various conditions.

EXPERIMENTAL PROCEDURE AND MATERIALS

Seeds

Kinds of Seeds Used.

Barley (Hordeum vulgare L.), Beecher variety, six-row, smooth, spring.

Corn (Zea mays L.), DeKalb, Yellow Hybrid.

Milo (Sorghum vulgare Pers.), Midland variety, combine type, yellow.

Oats (Avena sativa L.), Nemaha variety, white, spring.

Wheat (Triticum aestivum L.), Pawnee variety, Hard Red Winter (Class IV).

Moisture Content of the Seeds. Before fumigation of the seeds, the moisture content of various lots of each kind of seed was adjusted to 10, 11, 12, 13, and 14 per cent. The moisture content was increased by introducing a calculated amount of distilled water by pipette to seeds held at room temperature in a two-quart glass jar. The jar was then sealed with a two-piece Kerr lid and rolled a few minutes each day for one week to thoroughly mix the water and seeds. Moisture contents were decreased, when necessary, by drying seeds in a forced-air oven at 90° F. Moisture measurements were made by use of a Steinlite Moisture Tester. The manufacturer of this tester claims it has an accuracy of ± 0.25 per cent. Other investigators (Hlynka and Anderson, 1949) found the error of estimate to be ± 0.4 per cent. Since no method was available to control the atmospheric humidity in the laboratory, the moisture contents of the seeds were not maintained at their original levels after fumigation.

Fumigation of the Seeds

Seed samples were contained in small bags made of open-mesh rayon curtain scrim. Of the small grains, 100 grams of seeds were used in each bag; 50 grams of corn were used in each bag. Only one kind of seed was used at a time, and each moisture level was placed in a separate bag. Bags containing each moisture level of seeds were suspended by strings to the center of each 5-gallon glass fumigation bottle (Plate I). This made a total of 250 to 500 grams of seeds per bottle, depending on the kind of seed and number of moisture levels used in the test series. The ratio of seeds to air-space thus approximated that which would normally be found in a large warehouse partially filled with bags or small bins of seeds.

Methyl bromide was applied at the rates of 2, 4, 6, and 8 pounds per 1,000 cu. ft. in all test series and also at 0.6, 1, 3, and 5 lbs. in a few additional tests with wheat. The fumigant was measured at 0° F. in the liquid state by a cold pipette and was introduced into the fumigation bottle by releasing a partial (about 15 inches of mercury) vacuum created in the bottle just prior to fumigation. The air rushing through the pipette and stopcock and into the bottle to satisfy the vacuum greatly aided in vaporizing the liquid methyl bromide (B.P.=4.5° C.) and dispersing the gas in the bottle. The fumigations were conducted at prevailing atmospheric pressures, 80° F. and for exposures of 4, 8, 12, and 24 hours.

EXPLANATION OF PLATE I

Seeds held in small bags (A) were fumigated in 5-gallon glass bottles as shown. The glass stopcock (B) and neoprene stopper (C) were used to admit the fumigant and to close the bottle.

PLATE I

Gas Analyses

Methyl bromide concentrations were determined immediately before removal of the seeds from each fumatorium by means of a Gow-Mac, double-pass, four-filament, thermal conductivity gas analyzer. This unit was calibrated by the author so that the unbalance of the Wheatstone bridge (using an air reference) gave readings for methyl bromide on the electrometer in terms of ounces per 1,000 cu. ft., up to a maximum of 400 ounces. This widely used method of gas analyses is fully described by Phillips and Bulger (1953), Monro et al. (1953), and Phillips (1957).

Aeration of Seeds and Bottles

After removal from the fumatoria the seeds were aerated for 24 hours by exposing them to the laboratory atmosphere in the open-mesh seed bags which were used in the fumigation. A portion of each sample which was not used in the first germination test was held in a 4-ounce glass jar for later tests. Each jar was covered with a screen lid to permit further aeration of the seeds and also to prevent entry of insects.

The 5-gallon bottles were air-washed by means of a high velocity vacuum pump which drew fresh air through the bottles. Each bottle was tested for the presence of fumigant with a halide leak detector after aeration and again just before use.

Germination Tests

Germination tests were conducted at three different time intervals after fumigation so that immediate and delayed effects could be observed. These tests were started 1 day, 30 days, and 6 months after fumigation. Only whole, undamaged seeds were used for the germination tests. In starting germination tests the seeds of the small grains were counted by use of a vacuum seed counter (Plate II, Figs. 1 and 2). Corn seeds were counted by hand. Standard germination blotter paper was used as the substrate. Two Manglesdorf germinators (Plate II, Fig. 3) were used and, except in a few preliminary tests, the general germination technique and procedures followed those recommended by the U. S. Department of Agriculture (1952). Standardized seedling interpretations were made for one complete test series with wheat in which the seeds were evaluated as to "normal", "abnormal", or "dead." In all other tests seeds which showed any sign of embryonic development, however slight, were counted as "germinated." According to the standardized seedling interpretations, only normal seedlings are considered germinated (U.S.D.A., 1952). Sprout counts were made after 5 and 10 days' incubation for the standardized seedling series. Normal seedlings found in the 5-day counts were removed from the blotters while others were left in place for further incubation. Only a 7-day count was made for each of the samples where seedlings were not evaluated.

Mold growth was a problem, especially in samples which were severely injured by the fumigant. After it was determined that no apparent difference was caused in seed viability by its use, a suspension of Captan (N-trichloromethyl thio tetrahydrophthalimide) in water was applied to the germination blotters before placement of the seeds. This effectively reduced the amount of mold growth.

EXPLANATION OF PLATE II

Fig. 1. Seeds were counted and placed on wet blotters by use of a vacuum seed counter. The small jars with screen tops were used to hold the samples after fumigation.

Fig. 2. A close-up view of the seed counter head.

Fig. 3. These two Manglesdorf germinators were used for seed incubation.

Fig. 1.

Fig. 2.

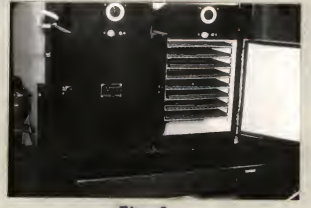


Fig. 3.

Replicates

Each fumigation was duplicated in nearly every test series. In some instances several replicate fumigation series were conducted. The numbers of seeds used in the germination tests ranged from 200 to 4,100 for each dosage, moisture, exposure and kind of seed. These numbers are indicated in the tabular data.

Tabulation and Statistical Evaluation of Data

Results of individual samples were calculated as percentages and analyzed by the analysis of variance. Least significant differences were computed at the five per cent level; these are indicated as "LSD*." In some instances, as when a sample was lost or insufficient seeds were available for a complete series, results for the missing individual samples were estimated by an unbiased method based on fewer replicates. This was done so that each series would have equal numbers of samples, thus simplifying the work involved in the analysis of variance. Wheat data, except the selected data, are expressed in terms of corrected differences between the treated samples and the checks. This correction was made by use of Abbott's formula to account for abnormal and dead seeds in the check samples.

RESULTS AND DISCUSSION

General

The data presented in this report reinforce, delineate, and extend the existing rather empirical knowledge of the facts relating to the effects of methyl bromide fumigation on seed viability. Injury to seeds

was found to be directly related to increases in (1) fumigant concentration, (2) length of exposure, (3) seed moisture content, and (4) length of the post-fumigation storage period. From other work (Cotton and Frankenfeld 1955_a and 1955_b), it was demonstrated that the degree of injury increased in close association with increasing fumigation temperatures.

The rates of increasing injury are not uniformly correlated with increases in each of the variable factors named above, but certain critical thresholds exist. These thresholds are so complexly interdependent that a minor change in one variable may cause the whole system to shift considerably.

Although seedling evaluations were not made for all of the kinds of seeds tested, it was apparent that many of the fumigated seeds were slow in developing and were often malformed. This was especially notable in the higher dosages and longer exposures. These observations were confirmed by the seedling evaluation tests with wheat.

The overall relative order of tolerance of the five kinds of seeds tested was: oats > barley > milo > corn > wheat (Plate III). It should be noted that this is the general order and does not hold true in every combination of variable factors.

Mold growth was definitely more troublesome in samples which were severely injured than in other samples. It was effectively controlled by the use of Captan.

EXPLANATION OF PLATE III

Fig. 1. An example of the effects of seed moisture content on germination after fumigation.

Fig. 2. An example of the effects of fumigant dosage on seed germination.

Fig. 3. An example of the effects of length of fumigant exposure on seed germination.

PLATE III

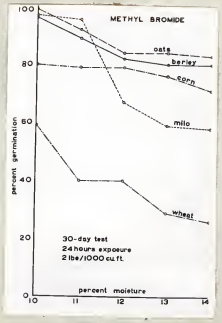


Fig. 1.

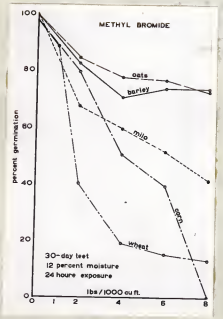


Fig. 2.

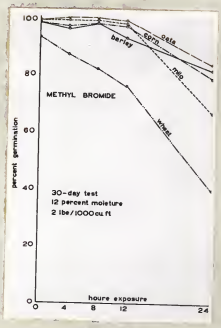


Fig. 3.

Other work, including that of Walkden and Schwitzgebel (1951) and of Strong and Lindgren (1957), which is still in progress, also points out the increased injurious effects of repeated fumigations and of the post-fumigation storage period.

Barley

Plate III illustrates the fact that barley was one of the more resistant kinds of seed. A summary and least significant differences are presented in Table 1. Examination of this table shows that there was a significant interaction among all the variables involved; namely, dosage x exposure x moisture x germination test number (the time interval between fumigation and the germination test).

A few examples are cited from Table 1 in the following tabulation. This tabulation shows the combinations of variables in which significant germination reductions first occurred. The comparisons are between treated samples and their respective checks (LSD* = 6.3). Other comparisons can be made by use of the proper LSD* value as indicated at the bottom of Table 1.

Table 1.—Beecher barley: Effects of methyl bromide fumigation[a] on seed germination.

Dosage and Length of Exposure	CH_3Br Analyses (Oz./M cu. ft.)	Number of Seeds Tested in Each Set of Conditions	Average Per Cent Germination[b] at Indicated Moisture Content of Seeds and Germination Test[c]									
			10 Per Cent		11 Per Cent		12 Per Cent		13 Per Cent		14 Per Cent	
			Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
2 lbs./1,000 cu. ft.												
Untreated Check	—	300	99.7	98.0	99.7	99.3	99.3	97.7	99.0	98.7	99.0	99.0
4 hours	27	500	99.4	97.8	99.4	99.2	99.2	96.4	99.0	99.2	98.4	97.0
8 hours	28	500	98.6	99.2	97.4	97.6	97.2	98.2	97.2	95.2	98.2	97.8
12 hours	29	500	97.8	98.2	98.2	97.8	93.4	93.4	90.0	91.2	93.0	87.2
24 hours	30	500	94.8	95.6	91.4	88.8	82.4	81.6	81.2	79.8	79.2	79.8
4 lbs./1,000 cu. ft.												
Untreated Check	—	300	98.3	97.0	100	99.3	97.3	96.7	93.3	87.7	90.7	88.3
4 hours	65	500	99.0	97.4	98.4	96.2	97.2	91.4	96.0	87.4	97.0	90.4
8 hours	62	500	98.8	93.4	95.8	84.2	93.4	77.4	87.8	74.4	92.0	74.4
12 hours	62	500	87.0	84.8	85.4	77.8	81.8	76.2	77.6	75.8	75.0	73.2
24 hours	62	500	72.8	72.8	74.6	74.6	75.0	69.8	75.2	77.8	71.0	72.6
6 lbs./1,000 cu. ft.												
Untreated Check	—	300	97.7	98.3	99.7	97.0	99.3	98.3	98.3	95.6	99.3	96.7
4 hours	99	500	98.4	95.4	98.0	93.2	95.8	94.6	92.6	85.0	91.8	84.2
8 hours	102	500	94.4	89.4	81.8	79.4	79.8	80.0	78.2	73.4	78.0	75.2
12 hours	99	500	86.8	86.4	79.8	81.2	76.6	79.6	80.0	73.0	71.2	72.0
24 hours	101	500	79.6	78.8	74.8	77.2	70.8	72.8	70.0	70.2	71.2	70.8

Table 1. (Concluded)

Dosage and Length of Exposure	CH_3Br Analyses (Dz./M cu. ft.)	Number of Seeds Tested in Each Set of Conditions	Average Per Cent Germination[b] at Indicated Moisture Content of Seeds and Germination Test[c]									
			10 Per Cent		11 Per Cent		12 Per Cent		13 Per Cent		14 Per Cent	
			Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
8 lbs./1,000 cu. ft.												
Untreated Check	—	300	99.3	97.0	98.2	97.0	98.7	95.3	96.0	95.0	98.3	94.7
4 hours	132	500	94.6	92.4	84.0	84.8	81.2	84.4	87.2	76.2	75.4	72.0
8 hours	128	500	85.6	80.6	72.8	71.6	74.8	79.8	76.0	77.4	65.8	57.2
12 hours	128	500	74.8	75.2	73.4	76.8	76.6	72.6	70.6	76.8	52.6	67.6
24 hours	127	500	74.2	74.2	71.2	73.2	71.6	73.4	68.8	73.2	45.6	49.2

Footnotes:

[a] The fumigation temperature was 80° F.
[b] All seeds showing embryonic development were counted as germinated.
[c] First germination test was started after fumigated seeds were aerated 24 hours. Second test was started after 30 days' aeration.

Least significant mean differences at the 5 per cent level (LSD*)
Comparing samples having 300 seeds, LSD* = 7.0.
Comparing samples having 300 seeds and samples having 500 seeds, LSD* = 6.3.
Comparing samples having 500 seeds, LSD* = 5.1.

Dosage (Lbs./M ft.^{3})	Exposure (Hours)	Moisture (Per cent)	First Test	Second Test
2	12	13	X	X
2	24	11	X	X
4	8	11		X
4	12	10	X	X
6	4	13		X
6	4	14	X	X
6	8	10		X
8	4	11	X	X
8	8	10	X	X

Except for a few instances, all combinations of conditions in which any one of the variables was greater than listed above also resulted in injury.

As indicated in column 2 of Table 1, gas analyses at the end of each fumigation showed that the fumigant concentrations were quite close to the amounts applied.

The results of the barley tests compare favorably with those found by other workers (Fisk and Shepherd, 1938; Mackie, 1938; Mayer and Gammon, 1957; California State Senate, 1955; and Lindgren et al., 1955).

Corn

Reference again to Plate III shows some representative germination values for corn after methyl bromide fumigation, and also indicates the relative susceptibility of corn as compared to the other species tested. As shown in Fig. 1 of Plate III, increasing the moisture content above 12 per cent caused a slight increase in susceptibility when the seeds were exposed to a dosage of 2 pounds per 1,000 cu. ft. for 24 hours. The 12 per cent moisture level is apparently the second moisture threshold, since the

treated seeds having only 10 per cent moisture germinated 80 per cent while the checks germinated about 99 per cent.

Fig. 2 of Plate III indicates that corn was very sensitive to increasing dosages. The dosage threshold for 12 per cent moisture and 24 hours' exposure lies some place below 2 pounds per 1,000 cu. ft. The exact point is not known because no dosages less than 2 pounds were tested in corn.

Fig. 3 of Plate III expresses the relationship between increasing lengths of exposure and damage to viability when the dosage was 2 pounds and the moisture was 12 per cent. Under these conditions the treatments were tolerated until exposures greater than 12 hours were used. The reaction of corn to increasing exposures was similar to that of oats, barley, and milo.

A summary and statistical statement are given in Table 2. The interaction among all the variables involved was not significant. Interactions of moisture x dosage x exposure and of germination test number x dosage x exposure were significant. The data were arranged according to the combinations just listed, and LSD* values were computed. The following tabulation cites examples of combinations of variables in which significant germination reductions first occurred. The comparisons are between treated samples and their respective checks.

Dosage (Lbs./M ft.^{3})	Exposure (Hours)	Moisture (Per Cent)	First Test	Second Test	LSD*
		(Moisture x Dosage x Exposure)			
2	24	10	(Combined)		8.1
4	8	10	do.		5.9
6	4	13	do.		8.1
6	8	10	do.		8.1
8	4	10	do.		8.1
		(Germination Test Number x Dosage x Exposure)			
2	24	(All combined)	X	X	5.3
4	8	do.	X	X	3.7
6	4	do.		X	5.3
6	8	do.	X	X	5.3
8	4	do.	X	X	5.3

Combinations of variables in which any one factor was increased above those listed resulted in increased injury. Some of the more severe treatments reduced germinations nearly to zero.

Thermal conductivity gas readings at the end of each fumigation indicated that even though the samples were not large, the seeds had sorbed a small amount of the methyl bromide during the 24-hour exposure periods. Gas readings at other times were well within the usual limits of variation (Table 2, column 2).

A series of tests was conducted by Frankenfeld in 1939 and reported by Cotton and Frankenfeld (1955_a). The results of Frankenfeld's work indicate that corn had a higher degree of tolerance than shown in this paper. The technique used by Frankenfeld differed from that used in these tests in that the ratio of corn to free-air space was much higher in the former, simulating that which is found in bulk storage. As mentioned earlier, in the tests reported here the ratio of seeds to free-air space approximated that which would be found in a large warehouse partially filled with bags or small bins of seeds. It is believed that this higher corn-to-air space ratio in Frankenfeld's tests caused a greater amount of the gas to be held by sorption, thus accounting for the higher tolerance indicated in the report by Cotton and Frankenfeld. Other factors, such as seed variety, may also effect these differences.

Table 2.—Yellow Hybrid Corn: Effects of methyl bromide fumigation[a] on seed germination.

Dosage and Length of Exposure	CH_2Br Analyses (Oz./M cu. ft.)	Number of Seeds Tested in Each Set of Conditions	Average Per Cent Germination[b] at Indicated Moisture Contents (1st and 2nd Tests Combined) 10 Per Cent	11 Per Cent	12 Per Cent	13 Per Cent	14 Per Cent	Number of Seeds Tested in Each Set of Conditions	Average Per Cent Germination[b] in Indicated Germination Test[c] (All Moistures Combined) Test 1	Test 2
2 Lbs./1,000 cu. ft.										
Untreated check	—	400	99.3	100	99.0	99.3	99.5	1000	99.7	99.1
4 hours	32	400	100	98.8	97.8	98.0	99.3	1000	99.1	98.4
8 hours	32	400	99.0	97.8	98.0	96.8	97.3	1000	99.1	96.4
12 hours	33	400	98.8	98.0	95.3	96.8	91.8	1000	97.2	95.0
24 hours	27	400	79.5	81.3	78.5	74.8	72.3	1000	77.5	77.0
4 Lbs./1,000 cu. ft.										
Untreated check	—	800	98.1	94.0	98.1	98.9	99.6	2000	98.0	97.5
4 hours	64	800	98.6	97.5	96.6	96.4	96.8	2000	97.6	96.8
8 hours	64	800	89.4	84.0	78.8	81.0	70.6	2000	78.7	82.9
12 hours	61	800	76.5	74.4	70.9	69.5	56.9	2000	69.3	70.0
24 hours	55	800	73.3	64.8	47.5	28.6	11.5	2000	45.2	45.0
6 Lbs./1,000 cu. ft.										
Untreated check	—	400	99.3	100	99.5	99.5	100	1000	99.8	99.5
4 hours	97	400	97.5	96.3	92.3	89.8	89.5	1000	94.9	91.2
8 hours	97	400	83.0	76.0	79.5	67.3	62.8	1000	77.5	69.9
12 hours	94	400	75.8	76.3	75.3	37.8	49.0	1000	69.3	56.3
24 hours	92	400	71.0	68.3	71.8	2.3	7.5	1000	46.2	34.1
8 Lbs./1,000 cu. ft.										
Untreated check	—	400	99.3	99.5	99.5	99.3	100	1000	99.8	99.2
4 hours	126	400	90.8	86.8	81.3	80.8	84.3	1000	87.6	81.9
8 hours	125	400	80.3	81.8	72.8	66.8	67.3	1000	77.4	73.1
12 hours	126	400	76.5	73.3	44.3	41.8	60.3	1000	72.5	45.9
24 hours	124	400	60.5	50.5	0.8	2.0	16.3	1000	31.9	20.1

Table 2. (Concluded)

Footnotes:

[a] The fumigation temperature was 80° F.
[b] All seeds showing embryonic development were counted as germinated.
[c] First germination test was started after fumigated seeds were aerated 24 hours. Second test was started after 30 days' aeration.

Least significant mean differences at the 5 per cent level (LSD*) when considering these interactions:

Moisture x Dosage x Exposure:
Comparing samples having 400 seeds, LSD* = 8.1.
Comparing samples having 400 seeds with samples having 800 seeds, LSD* = 7.2.
Comparing samples having 800 seeds, LSD* = 5.9.

Test Number x Dosage x Exposure:
Comparing samples having 1,000 seeds, LSD* = 5.3.
Comparing samples having 1,000 seeds with samples having 2,000 seeds, LSD* = 4.6.
Comparing samples having 2,000 seeds, LSD* = 3.7.

Milo

In general, milo appeared to be more tolerant than wheat and less tolerant than barley and oats. Plate III, Fig. 1 shows that sensitivity to 2 lbs./1,000 cu. ft. for 24 hours was greatly increased when seed moisture was increased above 11 per cent. When compared with corn under these conditions, the tolerance of milo was considerably above corn at moistures of 10 per cent and 11 per cent but dropped below corn at moistures of 12, 13, and 14 per cent. This indicates that at a 2-pound dosage and 24 hours' exposure milo was more sensitive to moisture changes than corn.

Figure 2, Plate III, shows that milo (12 per cent moisture and 24 hours' exposure) was more sensitive to increasing fumigant dosage than barley or oats, but was less sensitive than wheat or corn.

Increasing exposures (Fig. 3, Plate III), were tolerated by milo (12 per cent moisture and 2 pounds dosage) through 12 hours' exposure, but serious damage resulted from 24 hours' exposure.

The breaking points (thresholds) varied in relation to the combinations of all factors involved and may be found for each combination by studying Table 3. Analysis of variance revealed a significant interaction among the four factors involved, namely, moisture x dosage x exposure x germination test (a delayed effect). The LSD* was found to be 5.5 when comparing treated with untreated samples (Table 3). On the basis of this criterion, the following tabulation cites examples of combinations of variables listed in Table 3 in which significant germination reductions first occurred:

Dosage (Lbs./M ft.^{3})	Exposure (Hours)	Moisture (Per Cent)	First Test	Second Test
2	24	12	X	X
4	8	12		X
4	12	10	X	X
6	8	12	X	X
6	12	10	X	
8	4	11	X	X
8	8	10	X	X

Combinations of variables in which any one factor was increased above those listed resulted in increased injury. Results of gas analyses at the end of each fumigation indicated that the methyl bromide concentrations were approximately equal to the amount applied.

Experiments reported by Lindgren et al. (1955) indicate similar results as reported above.

Oats

Oats were more tolerant of methyl bromide than any other species tested, as shown in Figs. 1, 2, and 3, Plate III. Increasing the moisture, dosage, and exposure caused moderate increases in damage, but not to the extent observed with the other seeds tested.

Statistical analysis of the data disclosed that significant interactions occurred only at the 2-way level; namely, dosage x exposure, moisture x exposure, and dosage x germination test number. A summary of the individual tests, arranged according to the combinations having significant interactions, is given in Table 4. By using the appropriate least significant difference values which are listed at the bottom of Table 4, comparisons can be made in the table to show which treatments caused injury and also whether differences existed between first and second germination tests.

Table 3.—Midland milo: Effects of methyl bromide fumigation[a] on seed germination.

Dosage and Length of Exposure	CH_3Br Analyses (Oz./M cu. ft.)	Number of Seeds Tested in Each Set of Conditions	Average Per Cent Germination[b] at Indicated Moisture Content of Seeds and Germination Test[c]									
			10 Per Cent		11 Per Cent		12 Per Cent		13 Per Cent		14 Per Cent	
			Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
2 lbs./1,000 cu. ft.												
Untreated Check	—	300	97.3	99.7	97.7	98.7	99.3	99.0	98.7	99.0	98.0	98.7
4 hours	27	500	98.2	98.6	96.8	98.2	98.2	98.6	98.2	98.8	98.0	97.8
8 hours	28	500	98.4	99.4	98.6	98.6	97.6	99.6	99.0	99.0	95.4	97.8
12 hours	29	500	99.4	98.4	97.6	98.8	97.4	97.6	96.2	98.0	97.8	95.6
24 hours	30	500	97.4	96.6	94.6	96.2	70.0	66.8	63.2	58.8	57.6	58.2
4 lbs./1,000 cu. ft.												
Untreated Check	—	300	98.7	98.7	98.0	97.7	97.7	97.7	98.3	98.3	97.7	99.3
4 hours	65	500	99.0	99.0	99.0	99.0	98.4	97.8	99.2	96.8	98.6	98.8
8 hours	62	500	98.8	97.4	94.6	98.0	92.4	91.2	62.4	75.6	71.6	75.4
12 hours	62	500	90.0	91.8	65.8	85.6	57.8	64.4	40.4	54.0	57.6	50.4
24 hours	62	500	56.2	58.0	55.6	55.6	57.4	59.0	31.0	22.0	2.2	2.6
6 lbs./1,000 cu. ft.												
Untreated Check	—	300	99.0	99.7	99.3	98.3	99.7	99.3	99.0	98.7	98.0	99.7
4 hours	99	500	97.4	98.4	98.8	98.6	97.2	98.4	97.2	95.8	97.4	97.6
8 hours	102	500	98.2	97.6	96.4	96.4	70.2	80.6	54.4	60.6	45.8	48.8
12 hours	99	500	93.0	97.4	61.8	80.0	56.8	61.4	50.2	47.0	16.4	16.4
24 hours	101	500	56.0	56.2	52.4	56.0	51.6	51.4	0	1.6	0	0
8 lbs./1,000 cu. ft.												
Untreated Check	—	300	99.0	99.0	98.7	100	98.3	100	98.0	99.7	99.7	98.7
4 hours	132	500	95.2	97.4	90.0	94.8	90.4	94.0	87.2	85.8	67.0	79.2
8 hours	128	500	79.8	68.4	58.4	60.2	59.4	54.8	50.0	57.6	1.2	0.2
12 hours	128	500	57.8	62.4	58.4	61.6	50.8	57.2	20.2	18.6	0	0
24 hours	127	500	54.2	58.2	47.2	58.6	44.8	40.8	0.2	0	0	0

Table 3. (Concluded)

Footnotes:

[a] The fumigation temperature was 80° F.
[b] All seeds showing embryonic development were counted as germinated.
[c] First germination test was started after fumigated seeds were aerated 24 hours. Second test was started after 30 days' aeration.

Least significant mean differences at the 5 per cent level (LSD^*):
Comparing samples having 300 seeds, $LSD^* = 6.1$.
Comparing samples having 300 seeds with samples having 500 seeds, $LSD^* = 5.5$.
Comparing samples having 500 seeds, $LSD^* = 4.7$.

Table 4.—Nemaha Oats: Effects of methyl bromide fumigation[a] on seed germination.

Length of Exposure and Germination Test[b]	Number of Seeds Tested in Each Set of Conditions	Average Per Cent Germination After Indicated Dosage (Lbs./M cu. ft.)				Number of Seeds Tested in Each Set of Conditions	Average Per Cent Germination at Indicated Moisture Contents				
		2 Lbs.	4 Lbs.	6 Lbs.	8 Lbs.		10 Per Cent	11 Per Cent	12 Per Cent	13 Per Cent	14 Per Cent
Untreated check	3,000	99.7[c]	99.0	99.2	99.1	2,400	99.1[d]	99.4	99.6	99.1	99.0
4 hours	5,000	99.6	98.7	89.4	88.1	4,000	99.2	95.6	93.9	92.1	91.2
8 hours	5,000	99.4	89.4	82.1	78.7	4,000	95.2	87.3	85.5	85.5	83.4
12 hours	5,000	98.4	83.9	78.2	75.8	4,000	89.5	83.7	84.5	83.1	79.5
24 hours	5,000	86.7	72.5	71.7	64.6	4,000	77.9	77.2	77.2	74.8	60.9
First test	11,500	95.9[e]	86.4	83.6	81.1						
Second test	11,500	97.1	89.2	82.8	78.3						

[a] The fumigation temperature was 80° F.

[b] First germination test was started after fumigated seeds were aerated 24 hours. Second test was started after 30 days' aeration.

[c] All moistures and first and second germination tests combined for each value given in this section.

[d] All dosages and first and second germination tests combined for each value given in this section.

[e] All moistures and exposures combined for values given in this section.

Least significant mean differences at the 5 per cent level (LSD*) when considering these interactions:

Dosage x Exposure:

Comparing samples having 3,000 seeds, LSD* = 6.0.

Comparing samples having 3,000 seeds with samples having 5,000 seeds, LSD* = 5.4.

Comparing samples having 5,000 seeds, LSD* = 4.7.

Moisture x Exposure:

Comparing samples having 2,400 seeds, LSD* = 6.6.

Comparing samples having 2,400 seeds with samples having 4,000 seeds, LSD* = 6.0.

Comparing samples having 4,000 seeds, LSD* = 5.2.

Dosage x Test:

LSD* = 2.2.

The following tabulation cites those combinations of variables in which significant injury first occurred:

Dosage (Lbs./M ft.^{3})	Exposure (Hours)	Moisture (Per Cent)	LSD*
	(Dosage x Exposure)		
2	24	All combined	5.4
4	8	do.	5.4
6	4	do.	5.4
	(Exposure x Moisture)		
All combined	12	10	6.0
do.	8	11	6.0
do.	4	13	6.0

Each combination in which either factor was increased resulted in increased injury; those with smaller factors were not damaged. Relationships of germinations in the first tests (24 hours' aeration) and the second tests (30 days' aeration) were somewhat erratic, however, two definite trends are shown. The 4-pound dosage (all moistures and exposures combined) caused a significantly greater reduction in the first test than was found in the second test. The reverse situation was true for samples treated with the 8-pound dosage.

Table 5 lists average germinations for each combination of moisture x exposure x dosage x germination test number. Although the interaction at this level was not significant, it is obvious that the following are the approximate upper limits of tolerance:

Dosage (Lbs./M ft.^{3})	Exposure (Hours)	Moisture (Per Cent)
2	24	11
4	8	11
4	12	10
6	4	12
8	4	11

Table 5.--Nemaha oats: Effects of methyl bromide fumigation[a] on seed germination.

Dosage and Length of Exposure	CH_3Br Analyses (Os./M cu. ft.)	Number of Seeds Tested in Each Set of Conditions	Average Per Cent Germination[b] at Indicated Moisture Content of Seeds and Germination Test[c]									
			10 Per Cent		11 Per Cent		12 Per Cent		13 Per Cent		14 Per Cent	
			Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
2 lbs./1,000 cu. ft.												
Untreated Check	—	300	100	100	99.7	100	99.7	99.3	99.7	100	99.3	99.7
4 hours	35	500	99.2	100	99.8	100	99.6	100	99.8	99.2	98.6	99.8
8 hours	34	500	100	99.8	99.6	99.4	99.0	100	99.4	98.0	99.4	99.4
12 hours	34	500	100	100	98.4	99.8	98.4	99.2	96.2	98.0	95.4	98.6
24 hours	34	500	99.8	99.6	85.6	91.6	81.4	84.4	79.4	84.4	78.8	83.4
4 lbs./1,000 cu. ft.												
Untreated Check	—	300	97.7	99.0	99.3	98.3	98.7	100	99.3	99.7	99.3	99.0
4 hours	67	500	99.2	99.4	98.8	99.4	98.4	98.8	97.6	98.6	97.8	98.6
8 hours	68	500	98.8	99.2	89.8	97.0	83.6	88.8	83.8	85.2	84.8	82.8
12 hours	66	500	97.8	98.2	75.2	83.8	83.8	83.2	76.8	82.6	77.2	80.4
24 hours	67	500	72.6	73.6	74.0	74.4	75.0	77.4	65.4	80.0	60.8	71.4
6 lbs./1,000 cu. ft.												
Untreated Check	—	300	99.0	99.3	99.7	99.7	99.3	100	96.0	100	99.3	99.3
4 hours	98	500	99.0	99.0	93.2	89.8	94.6	88.2	89.2	86.0	85.0	88.0
8 hours	98	500	86.4	91.4	84.2	84.4	82.6	76.2	80.6	82.2	76.6	76.2
12 hours	96	500	76.2	79.0	78.6	82.2	80.2	78.0	85.0	76.8	73.2	72.8
24 hours	96	500	72.4	75.4	75.2	74.0	78.8	75.8	76.8	72.8	59.4	56.4
8 lbs./1,000 cu. ft.												
Untreated Check	—	300	99.0	99.0	99.7	99.0	100	100	99.0	99.0	99.3	96.7
4 hours	134	500	99.4	98.8	95.2	88.2	89.8	81.8	87.6	78.8	80.4	81.4
8 hours	132	500	96.4	89.8	69.6	74.6	78.8	75.0	78.0	76.6	74.4	73.4
12 hours	132	500	85.2	80.0	75.6	76.2	79.2	73.8	79.6	70.0	73.4	65.2
24 hours	125	500	73.8	69.2	70.0	72.6	72.0	72.4	70.4	68.8	39.6	37.6

Table 5. (Concluded)

Footnotes:

[a] The fumigation temperature was 80° F.
[b] All seeds showing embryonic development were counted as germinated.
[c] First germination test was started after fumigated seeds were aerated 24 hours. Second test was started after 30 days' aeration.

There are no significant interactions among the above data. Least Significant Differences are computed for those interactions which are significant and are presented in Table 4.

In fact, some of the examples just cited sustained moderate amounts of injury. In each case, an increase in one of the variables resulted in damage.

Gas analyses at the end of each of the exposures corroborate the theoretical dosages, since they lie within the usual limits of experimental error.

The results of the tests with oats agree with the preliminary findings of Fisk and Shepherd (1938) and those in the khapra beetle research (California State Senate, 1955).

Wheat

General. Wheat was definitely the most susceptible species tested. Several hundred seeds were used in each of the test series with wheat, partly because of its erratic response and also because wheat viability is of great economic importance. Flour millers do not want dead wheat. There is experimental evidence that shows germ damage from fumigation is directly associated with inferior bread quality when made from flour of damaged wheat (Cotton et al. 1946).

Figure 1, Plate III, illustrates the fact that the sensitivity does not always change in direct and uniform proportions to changes in moisture content. It appears that certain critical moisture thresholds exist here also which are, obviously, dependent upon the combination of the other variables involved. Similar thresholds undoubtedly occur for the other variables too, but in the combinations used for the data in Figs. 2 and 3, Plate III, they are not readily apparent. Figures 2 and 3 indicate that injury to wheat is more uniformly related to dosage and exposure than to moisture, under the conditions of these tests.

Interactions of Variable Factors. Graphic representations of the germination results from seeds treated with various combinations of dosage, moisture, and exposure, aid in giving a clearer understanding of these interactions. Figures 1, 2, and 3, Plate IV, present a few such combinations of summarized data.

The relationship of dosage x moisture x germination is illustrated in Fig. 1. During a 4-hour exposure, significant, but not drastic, reductions in viability resulted from treatments of 6-pound dosage x 14 per cent moisture and of 8 pounds x 10 and 11 per cent moistures. Severe injury was associated with the 8-pound treatment of seeds having 12, 13, and 14 per cent moisture.

Data showing the association of dosage x exposure x germination are portrayed in Fig. 2. Germination of wheat having 12 per cent moisture was significantly, but not drastically, reduced by treatments of 2 pounds x 8 hours and of 2 pounds x 12 hours. The only samples surviving without injury were those in treatments of 2 pounds, 4 pounds, and 6 pounds x 4 hours. All treatments other than those listed above caused great amounts of injury.

Interactions of moisture x exposure x germination are shown in Fig. 3. When fumigated with 2 pounds of methyl bromide per 1,000 cu. ft. slight to moderate damage was done to samples in treatments of 4 hours x 14 per cent moisture, 8 hours x 12, 13, and 14 per cent moisture, and 12 hours x all moistures. Extensive germination reductions resulted at all moistures x 24-hour exposure.

Summary of All Tests with Wheat. The results of all tests conducted with wheat are summarized in Table 6. All these data were not included in the statistical analyses because of the irregular and widely varying numbers

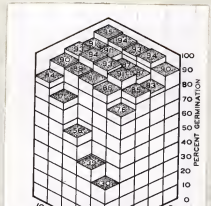
EXPLANATION OF PLATE IV

Fig. 1. Data illustrating the interactions of seed moisture content and fumigant dosage on germination of wheat after fumigation.

Fig. 2. Data illustrating the interactions of length of fumigant exposure and dosage on germination of wheat after fumigation.

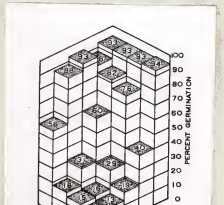
Fig. 3. Data illustrating the interactions of seed moisture content and length of fumigant exposure on germination of wheat after fumigation.

PLATE IV



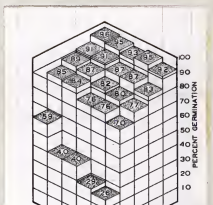
Germination of Pawnee wheat 30 days after fumigation with methyl bromide for 4 hours at 80°F.

Fig. 1.



Germination of Pawnee wheat 30 days after fumigation with methyl bromide at 80°F. The moisture content of the wheat was 12 percent.

Fig. 2.



Germination of Pawnee wheat 30 days after fumigation with 2 lbs methyl bromide per 1000 cu.ft. at 80°F.

Fig. 3.

Table 6.—Pawnee Wheat: Effects of methyl bromide fumigation[a] on seed germination. Summary of all tests conducted.

Dosage and Exposure	CH_3Br Analyses: (Oz./M cu. ft.)	Mean Per Cent Difference[b] Between Germination[c] of Fumigated and Check Samples at Indicated Germination Test and Moisture Content												
		One Day after Fumigation					30 Days after Fumigation					Six Months after Fumigation		
		10 Per Cent	11 Per Cent	12 Per Cent	13 Per Cent	14 Per Cent	10 Per Cent	11 Per Cent	12 Per Cent	13 Per Cent	14 Per Cent	10 Per Cent	12 Per Cent	14 Per Cent
0.6 lb./M cu. ft.														
24 hours	11	-1[d] (500)[f]	-[e]	1 (500)	-	0 (500)	1 (500)	-	2 (500)	-	1 (500)	-	-	-
1 lb./M cu. ft.														
4 hours	16	-4 (400)	-6 (500)	-6 (500)	-4 (500)	3 (500)	2 (500)	-1 (500)	-5 (500)	-3 (500)	-8 (500)	-	-	-
8 hours	15	-2 (500)	-6 (500)	-10 (500)	-1 (400)	0 (500)	1 (500)	-7 (500)	-6 (500)	-7 (500)	-6 (500)	-	-	-
12 hours	16	-6 (300)	-7 (500)	-4 (500)	2 (500)	4 (500)	-5 (500)	-5 (500)	-9 (500)	-5 (500)	-11 (500)	-	-	-
24 hours	16	-3 (900)	7 (500)	-6 (1000)	6 (500)	-3 (1000)	-1 (1000)	-9 (500)	-7 (1000)	-11 (500)	-9 (1000)	-	-	-
2 lbs./M cu. ft.														
4 hours	31	0 (2600)	-2 (2000)	-2 (2600)	-1 (2000)	1 (2600)	-4 (2600)	-3 (2000)	-5 (2600)	-7 (2000)	-9 (2600)	-2 (600)	-6 (600)	-9 (600)
8 hours	31	1 (2600)	-3 (2000)	-5 (2600)	-6 (2000)	0 (2600)	-6 (2600)	-7 (2000)	-11 (2300)	-15 (2000)	-15 (2600)	-3 (600)	-12 (600)	-15 (600)
12 hours	31	-3 (3100)	-23 (2500)	-18 (3100)	-22 (2500)	-19 (3100)	-11 (3100)	-12 (2500)	-18 (3100)	-20 (2500)	-24 (3100)	-6 (600)	-31 (600)	-34 (600)
24 hours	31	-32 (4100)	-57 (2500)	-56 (4100)	-70 (2500)	-62 (4100)	-39 (4100)	-58 (2500)	-57 (4100)	-69 (2500)	-72 (4100)	-41 (600)	-62 (600)	-70 (600)
3 lbs./M cu. ft.														
24 hours	46	-5 (1000)	-	-47 (1000)	-	-74 (1000)	1 (1000)	-	-41 (1000)	-	-71 (1000)	-	-	-

Table 6. (Continued)

Dosage and Exposure	CH_3Br Analyses: (Oz./M cu. ft.)	Mean Per Cent Difference[b] Between Germination[c] of Fumigated and Check Samples at Indicated Germination Test and Moisture Content												
		One Day after Fumigation					30 Days after Fumigation					Six Months after Fumigation		
		10 Per Cent	11 Per Cent	12 Per Cent	13 Per Cent	14 Per Cent	10 Per Cent	11 Per Cent	12 Per Cent	13 Per Cent	14 Per Cent	10 Per Cent	12 Per Cent	14 Per Cent
4 lbs./M cu. ft.														
4 hours	64	0 (2100)	-2 (1500)	-4 (2100)	-3 (1500)	-6 (2100)	-1 (2100)	0 (1500)	-1 (2100)	-2 (1500)	-8 (2100)	-3 (600)	-8 (600)	-21 (600)
8 hours	64	-7 (2100)	-22 (1500)	-42 (2100)	-45 (1500)	-55 (2100)	-8 (2100)	-19 (1500)	-36 (2100)	-44 (1500)	-53 (2100)	-10 (600)	-32 (600)	-40 (600)
12 hours	64	-31 (2600)	-61 (2000)	-70 (2600)	-81 (2000)	-83 (2600)	-28 (2600)	-57 (2000)	-69 (2600)	-78 (2000)	-82 (2600)	-25 (600)	-59 (600)	-76 (600)
24 hours	63	-65 (3600)	-83 (2000)	-81 (3600)	-87 (2000)	-86 (3600)	-64 (3600)	-81 (2000)	-80 (3600)	-83 (2000)	-87 (3600)	-70 (600)	-73 (600)	-73 (600)
5 lbs./M cu. ft.														
24 hours	82	-22 (1000)	—	-88 (1000)	—	-92 (1000)	-12 (1000)	—	-85 (1000)	—	-92 (1000)	—	—	—
6 lbs./M cu. ft.														
4 hours	95	-12 (1600)	-4 (1000)	-14 (1600)	-11 (1000)	-22 (1600)	-6 (1600)	-6 (1000)	-6 (1600)	-9 (1000)	-17 (1600)	-12 (600)	-13 (600)	-46 (600)
8 hours	99	-39 (2100)	-44 (1500)	-67 (2100)	-82 (1500)	-84 (2100)	-41 (2100)	-45 (1500)	-67 (2100)	-74 (1500)	-81 (2100)	-85 (600)	-81 (600)	-82 (600)
12 hours	97	-66 (2100)	-79 (1500)	-84 (2100)	-87 (1500)	-89 (2100)	-66 (2100)	-80 (1500)	-83 (2100)	-86 (1500)	-88 (2100)	-76 (600)	-82 (600)	-89 (600)
24 hours	96	-75 (3100)	-87 (1500)	-87 (3100)	-88 (1500)	-94 (3100)	-85 (3100)	-86 (1500)	-86 (3100)	-88 (1500)	-95 (3100)	-87 (600)	-86 (600)	-94 (600)

Table 6. (Concluded)

Dosage and Exposure	CH_3Br Analyses: (Oz./M cu. ft.)	Mean Per Cent Difference[b] Between Germination[c] of Fumigated and Check Samples at Indicated Germination Test and Moisture Content												
		One Day after Fumigation					30 Days after Fumigation					Six Months after Fumigation		
		10 Per Cent	11 Per Cent	12 Per Cent	13 Per Cent	14 Per Cent	10 Per Cent	11 Per Cent	12 Per Cent	13 Per Cent	14 Per Cent	10 Per Cent	12 Per Cent	14 Per Cent
8 lbs./M cu. ft.														
4 hours	130	-22 (2100)	-14 (1500)	-38 (2100)	-54 (1500)	-82 (2100)	-12 (2100)	-19 (1500)	-39 (2100)	-59 (1500)	-64 (2100)	-30 (600)	-45 (600)	-68 (600)
8 hours	128	-56 (2100)	-74 (1500)	-82 (2100)	-88 (1500)	-86 (2100)	-64 (2100)	-74 (1500)	-80 (2100)	-84 (1500)	-87 (2100)	-73 (600)	-80 (600)	-77 (600)
12 hours	128	-77 (2100)	-84 (1500)	-85 (2100)	-87 (1500)	-92 (2100)	-78 (2100)	-84 (1500)	-84 (2100)	-88 (1500)	-93 (2100)	-77 (600)	-78 (600)	-87 (600)
24 hours	123	-81 (3100)	-87 (1500)	-88 (3100)	-89 (1500)	-96 (3100)	-78 (3100)	-87 (1500)	-87 (3100)	-88 (1500)	-95 (3100)	-78 (600)	-79 (600)	-94 (600)

[a] The fumigation temperature was 80° F.
[b] Differences were corrected by Abbott's formula to account for dead seeds in checks.
[c] All seeds showing embryonic development were counted as germinated.
[d] Negative numbers indicate a lower value in the treated sample than in the check sample.
[e] No test made.
[f] Number of seeds tested in treated samples.

of seeds used in each different set of conditions. Representative data were selected for statistical evaluation. The results of these selected data are summarized in Table 7 and those of the seedling evaluations in Table 8, both of which will be discussed subsequently.

A study of the corrected germination differences between check and treated samples (Table 6) shows that a dosage of 0.6 pounds per 1,000 cu. ft. was tolerated by all samples, including those having 14 per cent moisture which were exposed for 24 hours.

The one-pound dosage caused slight injury in a few samples, with the maximum reduction being 11 per cent.

Two-pound dosages caused injury in proportion to the moisture content and the length of exposure. For example, seeds having 14 per cent moisture were injured as follows: (third germination test, 6 months after treatment) 4 hours' exposure, 9 per cent dead; 8 hours' exposure, 15 per cent dead; 12 hours' exposure, 34 per cent dead; and 24 hours' exposure, 70 per cent dead. Lesser amounts of damage resulted when the seed moisture content was lower; however, slight to extensive injury occurred in nearly every sample.

The treatment with a 3-pound dosage x 24-hour exposure was tolerated by the samples having 10 per cent moisture, but considerable damage resulted in samples having 12 and 14 per cent moisture.

Four-pound dosages were tolerated for 4 hours' exposure by all except 12 per cent (or more) moisture samples, which sustained slight to moderate injury. Eight-hour exposures resulted in viability reductions ranging from 7 per cent kill in the samples with 10 per cent moisture to 55 per cent kill in the samples with 14 per cent moisture. The ranges of damage resulting from 12- and 24-hour exposures to dosages of four pounds per 1,000 cu. ft.,

were 25 to 83 per cent and 64 to 87 per cent respectively. The degree of injury again was dependent upon the moisture content of the seeds.

Five-pound dosages caused from 12 to 92 per cent mortality of seed embryos.

Six- and 8-pound applications are not safe for wheat unless the moisture content is less than 10 per cent and/or the exposure is shorter than 4 hours.

Selected Data. Table 7, showing representative germination averages and LSD* values for wheat, was constructed to aid in a more detailed study of the data. The data used in this table were selected in units of entire series so that each set of conditions would be represented by the same number of replicates as every other series selected. It should be noted that in the 1-pound dosage series the germination was consistently lower in the first germination test than in the second. This difference was caused by inadvertant drying of the sprouting seeds.

The following tabulation cites examples in Table 7 in which significant germination reductions first occurred. The comparisons are between treated samples and their respective checks (LSD* = 7.7).

As a rule, combinations of variables having one factor of a greater value resulted in increased damage, and those with a smaller factor survived the treatment without injury. Other comparisons may be made in Table 7 by using the appropriate LSD* value. Except in the 1-pound treatment series, the delayed effect is indicated by comparing each pair (first and second) of germination tests.

Gas analyses showed that the fumigant concentrations were quite close to the calculated dosages.

Dosage (Lbs./M ft.^{3})	Exposure (Hours)	Moisture (Per Cent)	First Test	Second Test
1	8	12	X[1/]	
1	12	14		X
1	24	10	X[1/]	
1	24	11	X	X
2	8	12		X
2	12	11		X
2	12	13	X	X
2	24	10	X	X
4	8	11	X	X
4	12	10	X	X
6	4	11		X
6	4	14	X	X
6	8	10	X	X
8	4	10		X
8	8	10	X	X

Comparison with Results of Other Research with Wheat. The results of the wheat tests differ considerably from those reported by Cotton and Frankenfeld (1955$_b$) in that their data show the drier seeds to be more susceptible than those with 14 and 16 per cent moisture. A contrast of the two methods of fumigation may indicate the key to the differences in results. Frankenfeld fumigated seeds in one-quart containers which were fairly well loaded with wheat. The tests currently reported involved fumigation with relatively few seeds in 20-quart containers. Thus the ratio of commodity to air space was greatly different between the two experiments. It is commonly understood that the degree of fumigant sorption by the seeds is proportional to their moisture contents, i.e., the higher moisture is associated with greater sorption. It is possible, therefore, that the high moisture

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1/. These are questionable values because of inadvertent drying of seeds as explained in the text.

Table 7.—Pawnee wheat: Effects of methyl bromide fumigation[a] on seed germination. Summary and statistical statement of selected data.

Dosage and Length of Exposure	CH_3Br Analyses (Oz./M cu. ft.)	Number of Seeds Tested in Each Set of Conditions	Average Per Cent Germination[b] at Indicated Moisture Content of Seeds and Germination Test[c]									
			10 Per Cent		11 Per Cent		12 Per Cent		13 Per Cent		14 Per Cent	
			Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
1 lb./1,000 cu. ft.												
Untreated Check	—	300	85.0[d]	92.3	88.3	96.3	89.7	95.0	84.3	94.3	82.7	97.7
4 hours	16	500	81.2	94.0	81.6	95.2	83.8	90.4	78.6	90.8	80.8	90.2
8 hours	15	500	80.8	92.6	81.8	89.4	80.8	89.2	81.4	87.0	79.0	92.2
12 hours	16	500	80.6	87.0	80.8	91.4	84.8	88.0	84.2	88.8	82.6	87.4
24 hours	16	500	77.2	89.2	80.6	86.6	77.2	83.8	77.0	84.2	79.8	84.0
2 lbs./1,000 cu. ft.												
Untreated Check	—	300	100	98.6	98.7	96.0	98.3	97.3	99.0	99.3	99.7	93.7
4 hours	27	500	99.2	96.4	99.0	97.2	99.0	93.6	99.6	97.8	99.2	94.0
8 hours	28	500	98.8	95.6	98.2	96.0	97.8	89.4	97.6	93.6	98.2	95.2
12 hours	29	500	96.8	98.0	94.8	87.0	94.8	89.0	84.0	87.2	87.4	86.0
24 hours	30	500	63.4	66.0	26.0	27.2	29.8	27.2	23.2	24.4	29.6	18.6
4 lbs./1,000 cu. ft.												
Untreated Check	—	300	97.7	97.3	99.3	98.7	94.3	95.3	98.3	95.3	94.0	96.7
4 hours	65	500	97.2	96.7	96.8	96.4	96.7	96.8	98.2	96.0	91.0	90.0
8 hours	62	500	93.0	95.0	81.4	75.0	62.4	47.2	66.0	37.6	24.6	21.2
12 hours	62	500	85.2	89.4	32.8	27.8	15.2	10.0	7.4	8.0	5.6	4.6
24 hours	62	500	10.8	15.0	6.8	5.6	5.4	5.0	6.0	4.0	2.8	0.6
6 lbs./1,000 cu. ft.												
Untreated Check	—	300	99.3	96.0	98.0	95.7	98.7	96.3	99.7	93.7	97.7	96.7
4 hours	99	500	95.8	92.4	94.6	83.4	94.8	88.8	93.8	83.0	82.2	70.2
8 hours	102	500	91.6	83.8	66.4	61.0	32.0	21.4	18.4	11.8	7.4	5.6
12 hours	99	500	55.6	38.0	20.2	10.0	9.2	8.2	8.4	8.0	3.8	2.8
24 hours	101	500	4.8	7.8	5.8	5.0	4.6	4.6	4.6	4.8	1.2	2.2

Table 7. (Concluded)

Dosage and Length of Exposure	CH_3Br Analyses (Oz./M cu. ft.)	Number of Seeds Tested in Each Set of Conditions	Average Per Cent Germination[a] at Indicated Moisture Content of Seeds and Germination Test[c]									
			10 Per Cent		11 Per Cent		12 Per Cent		13 Per Cent		14 Per Cent	
			Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
8 lbs./1,000 cu. ft.												
Untreated Check	—	300	98.3	93.7	98.7	86.7	98.0	89.3	97.3	96.0	97.0	87.7
4 hours	132	500	94.0	78.4	85.0	44.8	70.4	32.2	56.8	11.4	17.8	5.4
8 hours	128	500	42.0	12.4	12.6	8.6	5.2	4.4	5.0	5.8	5.0	2.4
12 hours	128	500	9.2	5.5	6.2	6.6	5.0	5.0	3.8	3.2	1.8	0.6
24 hours	127	500	6.2	4.2	7.6	5.4	5.8	3.0	3.4	3.4	0.2	0.6

[a] The fumigation temperature was 80° F.
[b] All seeds showing embryonic development were counted as germinated.
[c] First germination test was started after fumigated seeds were aerated 24 hours. Second test was started after 30 days' aeration.
[d] Note that all samples in first test of the 1 lb. dosage showed lower germination than in the other tests. This was caused by inadvertent drying during incubation.

Least significant differences at the 5 per cent level (LSD*):
Comparing samples having 300 seeds, LSD* = 8.7.
Comparing samples having 300 seeds with samples having 500 seeds, LSD* = 7.7.
Comparing samples having 500 seeds, LSD* = 6.7.

seeds in Frankenfeld's tests sorbed and held methyl bromide in portions of the seeds other than the embryo, and thereby reduced the effective fumigant concentrations below levels toxic to the wheat embryos. If this were the case, the relatively few seeds in the tests reported here would not have had the sorptive capacity to demonstrate this theoretical phenomenon. Further research should be conducted to prove or disprove the theory because of the significant practical implications.

Fisk and Shepherd (1938) reported that a dosage of 10 pounds per 1,000 cu. ft. for 5 hours' exposure was tolerated by "dry" wheat seeds. Piper and Davidson (1938) wrote that fumigations which gave 100 per cent kill to five species of test insects (approximately 1 pound per 1,000 cu. ft. for 12 hours in atmospheric vault at 58 to 70° F.) had no effect on wheat viability.

In the khapra beetle research program (California State Senate, 1955), preliminary test results indicated that only slight injury resulted from fumigations of wheat under these conditions: 5 lbs. per 1,000 cu. ft., 12 hours' exposure, 10 per cent moisture, and 50 to 53° F.

The effects of repeated fumigations and of the time interval between fumigation and germination test were pointed out by Walkden and Schwitzgebel (1951). Wheat viability decreased more than 50 per cent in association with two fumigations and two years of storage. Untreated check wheat viability decreased only about 5 per cent. The fumigant used was a 3 to 1 mixture (by volume) of ethylene dichloride and carbon tetrachloride containing 10 per cent methyl bromide. Tests with the 3 to 1 mixture alone did not cause injury; in fact, it stimulated germination in some instances.

New Wheat. A few tests were conducted with wheat about one month after harvest. The response of these seeds was much more erratic than for aged

seeds. The tendency was for the new seeds to be more susceptible. Samples from this same lot of wheat about three months later responded similarly to those samples from other aged lots of wheat.

Seedling Evaluations. Table 8 presents a summary of the tests conducted for the purpose of determining sublethal and delayed effects. The mean per cent differences in the table express corrected differences between fumigated samples and their respective checks. Only seedlings which are normal are counted as germinated by official standards (U.S.D.A., 1952).

Injured seeds developed more slowly than untreated seeds. This is illustrated by the fact that corrected germination differences (normal seedlings) were smaller in the 10-day counts than in the 5-day counts. This means that many of the treated seeds had abnormal sprouts after 5 days' incubation which became normal by the time of the 10-day examination. The check samples were nearly completely normal after 5 days. Differences between 5-day and 10-day germination results were greatest in fumigated samples when final counts showed injuries were in the range of about 20 to 50 per cent reduction in 10-day normal seedlings.

Pronounced increases in the percentages of abnormal seedlings are shown in Table 8. These are especially notable when germinations (10-day normal seedlings) were reduced by 20 to 75 per cent below the check samples. The abnormal seedlings appeared stunted and/or twisted with many of them lacking essential parts. Frequently the tip of the plumule failed to emerge from the coleoptile, but continued to grow in the form of a loop. As a general rule, a small number of seeds survived the most severe treatments and developed normally, indicating that a few select individuals have a high degree of tolerance to methyl bromide. Perhaps a resistant strain could be developed by careful selection and breeding.

Table 8.—Pawnee wheat: Seedling evaluations to determine the effects of methyl bromide fumigation[a] on seed viability.

Dosage, Exposure, and Moisture	CH_3Br Analyses Oz./M cu. ft.	Mean Per Cent Difference[b] between Fumigated[c] and Check[d] Samples											
		1 Day after Fumigation				30 Days after Fumigation				6 Months after Fumigation			
		5-Day Count	10-Day Count			5-Day Count	10-Day Count			5-Day Count	10-Day Count		
			Normal	Normal	Abnormal	Dead	Normal	Normal	Abnormal	Dead	Normal	Normal	Abnormal	Dead
2 lbs./1,000 cu. ft.													
4 hours	31.8												
10%		2	2	-1[e]	-1	-5	-2	0	2	1	1	-3	1
12%		-4	0	-2	2	7	19[f]	-8	-8	-15	-10	3	6
14%		-6	-2	1	2	-2	12[f]	-5	-5	-23	-25	13	9
8 hours	31.0												
10%		0	0	0	0	-2	-1	0	1	-1	-1	-3	3
12%		-17	-2	1	1	-11	-8	-1	-6	-44	-43	29	11
14%		-14	-3	1	3	-20	-4	-3	7	-56	-53	32	7
12 hours	31.0												
10%		-24	-3	2	1	-43	-14	3	10	-50	-48	39	6
12%		-46	-18	10	7	-50	-23	6	14	-55	-55	20	32
14%		-43	-19	13	6	-62	-42	12	25	-74	-73	31	34
24 hours	29.0												
10%		-61	-29	15	13	-67	-64	7	52	-71	-72	18	46
12%		-67	-56	16	38	-68	-57	-3	54	-78	-78	11	62
14%		-71	-63	21	39	-75	-74	2	69	-77	-78	2	70

Table 8. (Continued)

Dosage, Exposure, and Moisture	CH_3Br Analyses (Oz./M cu. ft.)	Mean Per Cent Difference[b] between Fumigated[c] and Check[d] Samples											
		1 Day after Fumigation				30 Days after Fumigation				6 Months after Fumigation			
		5-Day Count	10-Day Count			5-Day Count	10-Day Count			5-Day Count	10-Day Count		
		Normal	Normal	Abnormal	Dead	Normal	Normal	Abnormal	Dead	Normal	Normal	Abnormal	Dead
4 lbs./1,000 cu. ft.													
4 hours	63.0												
10%		-20	-2	3	-1	-9	-7	0	7	-7	3	6	1
12%		-15	-8	2	5	-7	-8	0	7	-57	-42	31	8
14%		-25	-13	4	8	-40	-23	6	14	-66	-49	19	20
8 hours	64.0												
10%		-14	-4	0	4	-37	-8	-2	10	-62	-29	15	6
12%		-63	-37	10	25	-68	-56	21	33	-69	-62	25	32
14%		-57	-43	18	22	-71	-64	23	34	-72	-71	19	41
12 hours	62.3												
10%		-59	-28	12	15	-68	-45	19	23	-68	-67	34	25
12%		-65	-59	12	45	-76	-70	11	56	-77	-77	12	58
14%		-85	-76	1	70	-84	-82	1	76	-75	-76	-10	76
24 hours	60.0												
10%		-71	-69	1	64	-77	-76	-2	71	-74	-71	-12	69
12%		-78	-74	3	70	-81	-81	0	78	-74	-74	-4	73
14%		-88	-77	-4	77	-88	-85	-3	83	-80	-79	-6	72

Table 8. (Continued)

Dosage, Exposure, and Moisture	CH_3Br Analyses (Oz./M cu. ft.)	Mean Per Cent Difference[b] between Fumigated[c] and Check[d] Samples											
		1 Day after Fumigation				30 Days after Fumigation				6 Months after Fumigation			
		5-Day Count	10-Day Count			5-Day Count	10-Day Count			5-Day Count	10-Day Count		
		Normal	Normal	Abnormal	Dead	Normal	Normal	Abnormal	Dead	Normal	Normal	Abnormal	Dead
6 lbs./1,000 cu. ft.													
4 hours	95.5												
10%		-49	-30	-1	29	-63	-38	27	10	-81	-69	55	12
12%		-53	-33	-1	31	-59	-42	30	9	-77	-71	53	13
14%		-67	-49	-1	46	-79	-72	36	27	-73	-80	25	46
8 hours	103.0												
10%		-76	-72	2	63	-75	-74	14	56	-83	-82	4	74
12%		-79	-69	-1	62	-80	-80	10	64	-77	-80	6	70
14%		-82	-79	-8	80	-89	-85	-1	78	-86	-88	0	81
12 hours	100.5												
10%		-79	-78	-4	76	-85	-84	4	77	-83	-83	3	76
12%		-78	-77	-8	77	-80	-80	-5	79	-84	-84	-1	82
14%		-86	-81	-8	81	-91	-90	-3	85	-96	-96	-1	89
24 hours	97.0												
10%		-83	-82	-9	83	-84	-85	-3	85	-86	-86	-4	87
12%		-80	-77	-9	78	-87	-85	-5	84	-90	-90	0	86
14%		-96	-90	-5	87	-97	-96	-4	92	-96	-97	-4	94

Table 8. (Continued)

Dosage, Exposure, and Moisture	CH_3Br Analyses (lbs./M cu. ft.)	Mean Per Cent Difference[b] between Fumigated[c] and Check[d] Samples											
		1 Day after Fumigation				30 Days after Fumigation				6 Months after Fumigation			
		5-Day Count	10-Day Count			5-Day Count	10-Day Count			5-Day Count	10-Day Count		
		Normal	Normal	Abnormal	Dead	Normal	Normal	Abnormal	Dead	Normal	Normal	Abnormal	Dead
8 lbs./1,000 cu. ft.													
4 hours	129.0												
10%		-64	-59	-33	64	-68	-65	36	24	-72	-67	27	29
12%		-75	-73	6	65	-70	-68	26	37	-78	-78	29	45
14%		-76	-76	-3	73	-76	-74	4	61	-88	-80	1	69
8 hours	132.0												
10%		-64	-61	-43	74	-77	-75	-2	72	-74	-74	-14	73
12%		-79	-79	-2	78	-77	-76	-7	76	-81	-82	-1	80
14%		-89	-83	0	75	-90	-84	0	77	-93	-85	-2	79
12 hours	132.0												
10%		-68	-68	-43	78	-78	-78	-3	77	-70	-74	18	77
12%		-83	-82	-2	82	-78	-80	-8	81	-81	-80	-1	78
14%		-93	-85	-4	83	-94	-89	0	82	-98	-96	-2	87
24 hours	125.0												
10%		-69	-71	-19	75	-78	-78	-6	80	-77	-78	-3	78
12%		-81	-82	-2	82	-84	-82	-7	82	-84	-82	0	80
14%		-98	-93	-4	90	-98	-96	-2	92	-100	-98	-5	94

Table 8. (Concluded)

Footnotes:

[a] The fumigation temperature was 80° F.
[b] Differences were corrected by Abbott's formula to account for abnormal and dead seeds in checks.
[c] Six 100-seed samples were used for each set of conditions for the fumigated seeds.
[d] Except in a few cases, three 100-seed samples were used for each set of conditions for the check samples.
[e] Negative numbers indicate a lower value in the treated sample than in the check sample.
[f] For unknown reasons the check samples for these samples became very moldy and resulted in lower germination than the other checks or the treated samples.

As in the other series, gas analyses at the end of each fumigation showed concentrations closely approximating the calculated amount applied.

Discussion of Results and Relation of Seed Tolerance to Dosages Required for Insect Control

It should not be a surprise to learn that methyl bromide gas will damage or kill the seeds of our domestic plants in view of the fact that the fumigant is used as an effective means of controlling undesirable weeds such as bindweed and crabgrass. It will kill the seeds of these plants as well as the plants proper (Adamson, 1956). Of course, dosages used for such purposes are considerably higher than needed for stored-grain fumigation, and a margin of tolerance exists between treatments required for insect control and those which are lethal to seeds.

It is obvious that an absolute line of demarcation cannot be drawn to show the "safe" level of methyl bromide fumigation for insects infesting seeds. Rather, one should consider these factors: (1), dosage; (2), moisture content of the seed; (3), length of fumigant exposure; (4), kind of seed; (5), the post-fumigation storage conditions and period; (6), fumigation temperature; (7), history (previous fumigations, age, storage conditions, etc.) of the seeds; (8), ratio of commodity to total space in the fumatorium (i.e. sorption capacity); and (9), leakage factors in the fumatorium.

In general, for the first fumigation under carefully controlled conditions, it is possible to achieve satisfactory insect control without damage to seed viability. For example, Lindgren et al. (1955) reported methyl bromide LD_{95} values of 8.0 and 17.0 milligrams per liter at 70° F. for 24 hours' and 8 hours' exposures respectively for the khapra beetle larvae (Trogoderma granarium (Everts)), which is considered to be one of the more

resistant species. Dennis and Whitney (1955) found 2.7 and 5.1 milligrams per liter adequate to kill 100 per cent of adult rice weevils (Sitophilus oryza (L.)) and confused flour beetles (Tribolium confusum Duv.) respectively when fumigated at 70-80° F. for 20 hours' exposure in cylinders without the presence of a commodity.

Other laboratory tests by Whitney (1956) in grain-filled recirculators at 73-78° F. indicate that the following dosage rates are adequate to kill all stages of the rice weevil and the confused flour beetle adults, when exposed 24 hours: (1) In wheat, 10 per cent moisture: 5 milligrams per liter; 12 per cent moisture: 7.5 mg./l.; 14 per cent moisture: 7.5 mg./l. (2) In corn, 11.3 per cent moisture: 7.5 mg./l. (3) In milo, 14 per cent moisture: 12.5 mg./l. (4) In barley, 11 per cent moisture: 7.5 mg./l. and (5) In oats, 12 per cent moisture: 7.5 mg./l. Naturally, the interstitial gas concentrations were initially higher than the calculated dosages because of displacement and soon became lower because of the sorption factor.

Thus it is shown that the minimum treatments necessary for insect control may be used without expectation of injury to seeds. The margin of tolerance, however, may be quite narrow, and such factors as repeated fumigations and low initial viability may result in unexpected damage. Further testing is in progress.

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EFFECTS OF METHYL BROMIDE FUMIGATION ON THE VIABILITY OF BARLEY, CORN, MILO, OATS AND WHEAT SEEDS

by

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Methyl bromide is a highly efficient fumigant, which is widely used for the fumigation of stored grain, mills and warehouses. One of its undesirable qualities with respect to seed fumigation is its phytotoxicity. Viability of seeds may be seriously impaired by excessive treatments with methyl bromide.

Tests were conducted to determine the effects of methyl bromide on the viability of barley, corn, milo, oats and wheat seeds when fumigated at 80° F. with different combinations of dosage, exposure, and seed moisture content. Gas analyses were made at the end of each fumigation to verify the methyl bromide concentrations. Germination tests were conducted 24 hours, 30 days, and 6 months after fumigation to observe immediate and delayed effects. There was a pronounced decrease in viability in some cases with the increased post-fumigation period. In some experiments standardized seedling evaluations were made so that sublethal, injurious effects could be observed. Many of the fumigated seeds sprouted but did not develop normally.

A margin of tolerance usually exists between the dosages required for insect control and those which are injurious to high-quality, dry seeds. This margin of tolerance is dependent upon the complex interaction of several variable factors, including (1) the fumigant dosage applied, (2) the seed moisture content, (3) the length of exposure, (4) the kind of seed, (5) the post-fumigation storage period and conditions, (6) the fumigation temperature, (7) the history of the seed (age, previous fumigations, etc.), (8) the ratio of commodity to total space in the fumatorium (the sorption capacity) and (9) leakage factors in the fumatorium.

In general, the results of the study show that little or no injury occurred when the following combination of conditions existed: (1) the

seed moisture was less than 12 per cent, (2) the dosage was less than 2 pounds per 1,000 cu. ft., (3) the exposure period was less than 24 hours, and (4) the temperature was 80° F. High temperature, moisture, dosage, and long exposure all contribute to seed injury from fumigation. When combinations of fumigation conditions occur in which one (or more) of these variables is of a higher order than named above, moderate to extensive germination damage may be expected.

The over-all relative order of tolerance of the five species tested was oats > barley > milo > corn > wheat.