

THE EFFECT OF VARIOUS CHEMICALS ON THE  
RESPIRATION AND STORAGE BEHAVIOR OF WHEAT

by

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B. A., Evansville College, Evansville, Indiana, 1948

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A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Milling Industry

KANSAS STATE COLLEGE  
OF AGRICULTURE AND APPLIED SCIENCE

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## INTRODUCTION

Investigations concerning the beneficial effect of chemicals upon the storage characteristics of wheat might properly be divided into two main categories. The first classification would cover those studies which are concerned with the stimulation of two inherent physiological processes in the living seed which might increase its value for milling purposes. The second category would include those investigations which have to do with the prevention of the deleterious effects of storage that result from the operation of extrinsic agencies, such as excessive moisture, rodents, fungi, and insects.

This study has included some phases of both of these problems. Ethylene, a gas of marked physiological potency for some plants, was investigated for the possible improving or maturing effect that it might have on wheat or wheat products in storage. Numerous other compounds were investigated to determine their efficiency in inhibiting the deterioration of stored grain due to mold growth.

Any procedure which would accelerate the normal maturing or aging process of freshly harvested wheat would be of great value to the milling industry and to farmers. No longer would the harvesting period be determined solely by the stage of maturity of the wheat in the field. Within limits of considerable latitude, grain could be harvested when the proper equipment was readily available and when the weather and other con-

ditions were at their optimum for efficient harvesting. Then, by proper treatment in the mill or in the elevator, the wheat could be brought to the stage of maturity normally attained by natural aging in storage which makes for maximum efficiency in milling and baking.

The economic value of preservatives for wheat in storage may be emphasized by pointing out that at least 65,500,000 metric tons (72,200,000 short tons) of cereal grains, pulses and oil seeds are destroyed yearly insects, rodents, and fungi. This figure, which was stated by representatives at the International Meeting on Infestation in Foodstuffs sponsored by the Food and Agriculture Organization of the United Nations, held in London in August 1947, is approximately equal to the amount of these foods which enter world trade annually. Any method which could be used to minimize this tremendous loss of food would not only be of great economic advantage to the nations and individuals engaged in the production of such food, but would also help to improve the now sub-optimal diets of much of the world population.

#### REVIEW OF THE LITERATURE

##### Ethylene as a Maturing Agent

There are many references in the literature to investigations of the effect of ethylene on fruits, seedlings, etc.



Crocker (1943) states that ethylene is physiologically active in smaller concentrations than any other chemical. The gas will induce leaf epinasty in the African marigold if present to the extent of 0.001 p.p.m. of air. It hastens ripening changes in fruits and has been used commercially for this purpose. The changes which it induces seem to be mainly hydrolytic, e.g. starch to sugar. Many, if not all, respiring plant tissues produce ethylene. No mention is made in Crocker's book of any fungi-inhibiting or stimulating powers of ethylene.

The literature concerning the effect of ethylene on dormant grains is meager. Balls and Hale (1940) attempted to hasten the after-ripening of new wheat by treating it with ethylene. They claimed that the gas had a definite improving effect. Flour milled from ethylene-treated wheat produced bread which was of excellent texture and good color, and which was apparently normal in all other respects. On the other hand, bread made from the untreated control had smaller volume, was soggy and was "green" in color. Balls and Hale also found that germination was increased by small dosages of ethylene and decreased by large dosages of ethylene. Treatment with the gas apparently had no effect on rates of oxygen consumption by the grain.

Sundberg (1949) undertook an extensive series of experiments with ethylene-treated wheat of varying degrees of maturity. His results were largely inconclusive, although it appears that ethylene treatment decreased somewhat the breadmaking quality of flour milled from wheat harvested at 50 per cent moisture.

It decreased germination in some cases and increased it in others.

Part of the present study is a continuation of Sundberg's work with particular emphasis on the effect of ethylene on the respiratory characteristics of wheat harvested at different stages of maturity.

#### Grain Respiration as Related to Moisture Content and Mold Inhibitors

The respiration of grain seems to have afforded a popular field of investigation for biochemists interested in the cereal industries.

As early as 1891 Mountz discovered that the respiration of grain increased as the moisture content was raised. He found this increase to be slight until a moisture content of 13 to 14 per cent was reached, after which it increased much more rapidly. Sealing the grain in air-tight containers decreased its carbon dioxide production. Duvel (1904) similarly found that respiration of wheat increased as its moisture content was increased. A pronounced lowering of the seed viability accompanied this increase in respiration.

Bailey and Gurjar (1913) investigated many of the factors influencing the respiration of wheat. Grain which had been moist for a comparatively long time as a result of natural dampening gave a higher respiration rate than wheat which had been artificially dampened only three days previously. Shriv-

elled wheat was found to have a higher respiratory rate than sound wheat at the same moisture content. The maximum rate of respiration occurred at 35° C. Respiration was reduced by accumulated carbon dioxide, and respiration in an oxygen-free atmosphere was reduced to about two-fifths that in air.

It appears that these earlier investigators assumed that the increase in respiration following an increase in moisture content was due to a stimulation of the metabolic processes in the dormant seed. However, Gilman and Barron (1930) showed that heating and deterioration in stored corn was largely due to the effects of mold growth. These phenomena are associated with an increase in respiration. Semenik and Gilman (1944) stated that "the conditions under which deterioration .... (in corn) ... occurs and the changes which follow its initiation indicate that it is primarily a biological decomposition."

The connection of microorganisms with the heating phenomenon in various stored foodstuffs had been suggested much earlier. As early as 1912, Peirce had stated that microorganisms had much to do with rise in temperature of stored seeds, and, even before this, Miele (1907) had observed the presence of molds and bacteria in heating hay.

Milner and Goddes (1946) found that an intimate relationship exists between the increased mold growth and the increased respiratory rates in wheat at high moisture contents. The interdependence of these two phenomena was clearly demonstrated by Milner, Christensen and Goddes (1947) when they showed that



certain mold inhibitors have a depressant action on the respiration of moist wheat. They investigated 107 compounds known to have fungistatic action and found that thiourea and hydroxyquinoline sulfate were the most effective of these in inhibiting the respiration of damp grain.

Milner, Christenson and Geddes (1947a) found that the development of fat acidity in grain was a good indicator of the extent of mold growth.

Larbour, Clayton, and Wrenshall (1935) measured the carbon dioxide production of wheat at 20 per cent moisture content stored under various conditions. They found that carbon dioxide production increased much more rapidly in samples which were continuously aerated and that the initial respiration was also greater in these samples. They found that the accumulation of carbon dioxide in damp wheat tends to retard the respiration process and therefore they believe that the discontinuous process of respiration measurement does not give the maximum rate of carbon dioxide production for a given moisture content and temperature. Furthermore, it was observed that with the discontinuous method the rate of carbon dioxide production depended to some extent upon the free air space in the vessel; weight per bushel and degree of packing the sample would affect the rate of carbon dioxide production in small containers. Thus these experimenters decided that for comparative work the continuous method seemed less subject to error and accordingly it was adopted.

Larnour, Clayton and Marshall treated a number of their samples with various chemicals to determine the effect of mold inhibitors on the respiration rate. Samples which had been surface disinfected with mercuric chloride were aerated in the normal manner and also with air containing toluene vapor (about 1 cc in 24 hours). They found that the toluene vapor stimulated respiration above that of the control sample until the third day when a decrease began. The sample treated with mercuric chloride, but not with toluene, showed a constant decrease in respiration until the twelfth day, at which time a rapid decrease ensued. Final respiration rates of this sample (on the seventeenth day) was almost twice the maximum attained by the toluene treated sample and over thirty times that of the toluene-treated sample on the same day. They suggest that the initial stimulation of seed respiration by the toluene vapor was due to the effect of the chemical upon the metabolic processes of the embryo, and that the rapid increase in respiration on the twelfth day of the sample which had not been treated with toluene was due to mold growth.

These experimenters treated wheat at various moistures with continuous carbon tetrachloride vapor in air and compared the respiration rates with those of samples which were not treated with this chemical. They found that continuous application of carbon tetrachloride vapor prevented mold growth at 22 per cent moisture but not at 24 per cent moisture. There was observed a slow decrease in respiration at fairly high moisture contents

and they suggest that this was a result of the anaesthetic effect of carbon tetrachloride on the wheat metabolism. They conclude that the vapor of this chemical raised the critical moisture for rapid mold proliferation by at least two per cent. As a correlated observation, these workers found that wheat stored at 25 per cent moisture and treated with carbon tetrachloride showed no tendency to heat for 25 days, the duration of the experiment. The damp wheat stored for 25 days after treatment with carbon tetrachloride showed no diminution in baking quality.

Larmour and Bergsteinsson (1933) later extended this work and included such baking data on samples stored for as long as 40 weeks at various moisture contents. They employed five-pound samples of hard wheat stored at 31° C. It was found that samples at 12 per cent moisture which had been treated with as much as 20 cc of carbon tetrachloride showed no deleterious changes after 40 weeks of storage. Wheat stored at 18 per cent moisture became musty after 10 weeks in storage if the sample was treated with one cc or less carbon tetrachloride. The baking quality of all samples at this moisture level deteriorated after 20 weeks, regardless of the dosage of chemical employed. After four weeks, the samples stored at 24 per cent moisture exhibited a sour odor but no mustiness. All treated samples containing this percentage of moisture showed distinct evidence of damage to quality after four weeks, while the control was undamaged. The damage was slight in the sample treated with one cc of

carbon tetrachloride but severe with larger dosages. The damage increased with time, and after 20 weeks storage all samples were heavily damaged.

Altschul (1949) studied the relative effectiveness of various chemicals in decreasing the deterioration of cottonseed at high moisture contents. He did not attribute this deterioration to mold growth, but since he used the fat acidity values and the rate of heating as an indication of the rate of decomposition it may be assumed that the effects which he noted were due to fungi. He listed about 50 compounds which he had found to be effective in preserving cottonseed.

Whelton, Phaff, Hawk, and Fisher (1946) found that epoxides were powerful reagents against certain yeasts, molds and bacteria that attack foods. At 86° F., one ml of a 20 per cent ethylene oxide mixture per liter of air for three hours was necessary for complete killing of yeasts and molds. The fumigant was more effective as the temperature increased from 50° to 100° F. Propylene oxide was not as effective as ethylene oxide.

Sheffer and Duncan (1945) found that benzaldehyde, 2-chlorophenylhydrazine, ethyl mercurichloride and o-chlorophenol were quite effective in inhibiting mold growth on many substrates.

The present mold inhibitor studies were instituted with a view to extending the work of Milner, Christenson and Geddes, as well as that of Altschul, particularly in evaluating the effect of chemicals on the respiration of damp wheat and their effect on the commercial quality and baking and milling value



of the grain.

## INITIALS AND METHODS

### Description of the Wheat Samples Used

Several different samples of wheat were used in these studies. The first respirometer trial utilized a commercial mill mix of indefinite composition from the Kansas State College mill. This sample of grain is identified as wheat "A" in the subsequent paragraphs.

For screening the effectiveness of various compounds as mold inhibitors, a sample of COT (Comanche-Oro-Terrara) variety wheat supplied by the Kansas Agricultural Experiment Station at Hays, Kansas was used. This wheat appeared to be quite clean and free from foreign material. After being passed once through the experimental cleaner at Kansas State College, it was stored at 50° F. until used. It will be referred to in the following sections of this paper as wheat "B".

The work dealing with the effect of ethylene on mature and immature wheat utilized samples taken from a plot of Pawnee variety of the 1949 crop grown on the Kansas State College Agronomy Farm at Manhattan. Beginning in the early stages of filling, a few heads were picked almost daily to determine the moisture value and thus follow the course of maturation. This information, together with certain meteorological data for the



period covered, is given in Table 1.

All the wheat secured for ethylene studies prior to the first combine harvest on June 24 was obtained by hand picking the desired amount of wheat heads. When clean kernels only were required, as for the germination tests, the heads were hand threshed and the grain dried at room temperature, unless otherwise specified. The June 13 harvest was threshed on a small experimental thresher at the Agronomy Farm. Samples of wheat from the June 13 harvest will be designated by the letter "C" in the data tables.

The sample of mature wheat taken on June 24 was harvested by a combine. The grain was put through the experimental cleaner twice to remove foreign material and then was stored at room temperature until used. Wheat taken from this lot will be identified by the letter "D".

In July, a large sample of Pawnee seed wheat grown at Clay Center, Kansas was secured for use in storage studies and various other experiments. This wheat had been harvested in June of 1949 and was clean and of good appearance. The letter "F" will identify this wheat in the following paragraphs.

The source and description of the wheat samples used in these studies is given in Table 2.

#### Analytical Methods

Germination percentages were determined by the Seed Labora-

Table 1. Moisture changes in maturing Pawnee wheat and meteorological data for period of maturation.

Date : 1949 :	Moisture : content :	Precipitation : inches :	Temperature : range :	Wind : direction :	Remarks :
June 7	49.8	0.02	85	83	Single stage moisture on wheat heads
June 8	51.7	.10	73	64	Same
June 9	43.6	.08	79	64	Same
June 10	53.4	.23	81	63	Same
June 11	45.1	Trace	85	63	Same
June 12	45.2	Trace	87	70	Same
June 13	47.4	Trace	85	66	Same
June 14	43.0	.50	80	65	Two stage moisture on kernels
June 15	37.9	None	78	64	One stage moisture on wheat heads
June 16	36.2	None	83	65	Same
June 17	28.2	None	85	66	Same
June 18	25.3	.13	89	71	Two stage moisture on kernels Sample "C" harvested this day
June 19	--	Trace	83	70	No sample harvested
June 20	18.7	None	86	72	Two stage moisture on wheat heads
June 21	14.6	None	86	71	One stage moisture on wheat heads
June 22	12.7	None	90	64	Two stage moisture on wheat heads
June 23	12.3	None	94	71	One stage moisture on wheat heads
June 24	14.2	.52	85	70	One stage moisture on kernels Sample "D" harvested this day

Table 2. Identification and description of wheat samples used in experiments.

Sample : Designation:	Purpose	Date of : harvest :	Variety and other description
"A"	Preliminary respiration trials	1948 crop	Commercial mill mix
"B"	Preliminary respiration trials and screening fungistatic compounds	1948 crop	CGT grown at Hays, Kansas
"C"	Ethylene studies	June 18, 1949	Pawnee grown at Manhattan
"D"	Ethylene studies	June 24, 1949	Pawnee grown at Manhattan
"E"	Storage studies	1949 crop	Pawnee grown at Clay Center, Kansas

tory of the Kansas State Board of Agriculture at Manhattan. If the seeds were prechilled before germination, it is so indicated in the data tables.

Protein, ash, maltose and free fatty acids were determined in accordance with the procedure set forth in Cereal Laboratory Methods (1947). Free fatty acid values are expressed as milligrams of potassium hydroxide required to neutralize the acids in 100 g dry matter.

Daily moistures for samples taken from the field of maturing Pawnee wheat, as given in Table 1 and elsewhere, were determined by drying the whole wheat heads at 130° C. for one hour, unless otherwise specified. Moisture determinations on other samples were conducted in accordance with the procedures outlined in Cereal Laboratory Methods. The term "moisture", when used in this paper, indicates a moisture percentage determined by the one stage oven method, and the term "two-stage moisture" indicates a moisture percentage determined by the two-stage oven method. Results are expressed as percentage of sample and weight.

Perinograph tests were made in accordance with the methods suggested by the Brabender Corporation.

#### Baking Procedures

Baking tests were performed on the ethylene-treated wheat using a sponge procedure and the following formula:

700 g flour (as-is basis)

14 g salt

35 g sucrose

14 g yeast

21 g shortening

Baking tests on the wheat used for storage studies were conducted in accordance with a straight dough procedure. The following formulas were used for this group of tests:

Basic formula:

100 g flour

2 g yeast

5 g sucrose

2 g salt

3 g shortening

0.5 g malted wheat flour

The "brannte" formula consisted of the basic formula plus 0.5 g Arkady, and the "rich" formula consisted of the basic formula plus 0.5 g Arkady and 3 g dry milk solids. The absorption percentage listed in the data tables indicates the weight of water used in each mix expressed as percentage of the flour on an as-is basis.

Respirometer Experiments

The apparatus used for respiration studies on wheat is similar to that described by Milner and Geddes (1945). The



samples of grain are held in a thermostated bath at 30° C. and aerated with approximately two liters per day of atmospheric air which had been passed through a soda-line tower and a calcium chloride drying tube. No attempt was made to maintain the incoming air at a relative humidity in equilibrium with the moisture content of the wheat since experience showed that the grain lost very little, if any, of its moisture during the few days it was under study. Carbon dioxide and oxygen percentages were determined by means of a modified Haldane-Menderson gas analysis apparatus. The samples were maintained in the respirometer for varying periods, the duration of which was dependent upon the possibility of further information being secured from continued determinations. Values for carbon dioxide volume were converted to approximately standard temperature and pressure by multiplying the raw values by the factor 0.98.

Wheat was conditioned to the moisture contents required by placing the wheat in a large tin can, adding the necessary amount of water, shaking thoroughly and then storing overnight at room temperature.

#### Preliminary Screening Tests for Mold Inhibitors

Preliminary screening tests to indicate the probable efficiency of mold inhibiting compounds were performed in the following manner. Fifty grams of wheat at about 20 per cent moisture were placed in small screw-capped glass bottles and

treated with approximately 0.1 cc of the liquid chemicals and of 0.1 g of the solid chemicals. The bottles were then sealed and vigorously agitated. They were stored at room temperature and examined often for visual evidences of mold growth. After two weeks the bottles were opened and the contents air-dried at room temperature. Samples from each bottle were submitted for germination tests and the rest of the contents were used for other tests. One or more control bottles of untreated wheat were included as controls in each series.

#### Method for Determining the Toxicity of Propylene Oxide to Wheat

The tests to determine the toxicity of propylene oxide to wheat were performed by placing a 100-g sample of wheat in a tin can with a tight friction lid, adding the required amount of chemical, agitating thoroughly, and storing at room temperature. Samples were withdrawn at convenient times for germination tests.

#### Large-Scale Storage Tests

The storage tests utilized samples of wheat stored in one gallon glass jars with screw caps. After conditioning to the required moisture content, the wheat was placed in a blender and the chemical was added to it. After thorough mixing the grain was placed in four glass jars which were then stored at

room temperature. The jar caps were provided with a small hole, ordinarily closed with a rubber stopper, through which a glass tube could be inserted to aerate the contents with oxygen or nitrogen. Aeration with the appropriate gas was performed once each week. Sufficient gas was used to flush thoroughly the old atmosphere from the jar.

At four-week intervals, one jar from each set was removed and the contents subjected to tests for moisture, fat acidity and germination. A sample of the wheat was sent to the Production and Marketing Administration of the United States Department of Agriculture at Kansas City, Missouri, for commercial grading. After being examined for appearance and odor, the remaining portion of the wheat was milled on a Bahler experimental mill.

A sample of the flour obtained from the milled wheat was tested on the farinograph and the absorption and valorimeter values thus secured were noted. After noting the odor of the flour, it was baked into bread using a straight dough pup loaf procedure and the formulas which have been listed previously. The volume and weight of the loaves obtained was measured, and from these figures the specific volume was calculated using the simple formula: loaf volume  $\div$  loaf weight = specific volume. The external appearance of the loaves was graded relative to the other loaves in the same bake with special attention being paid to the break and shred, and the color, contour, size, and symmetry of the loaves. After cutting, the internal character

of the leaves was graded for grain and texture on an arbitrarily fixed absolute scale, and the odor and color were noted.

Except where otherwise noted, the mixtures of ethylene in air were made by drawing the undiluted ethylene into a displacement bottle first and subsequently drawing in sufficient air to provide the proper dilution.

## EXPERIMENTAL

### Ethylene Studies

The Effect of Ethylene on the Respiration of Immature Wheat Heads. The purpose of this experiment was to study the influence of ethylene on the respiration of immature whole heads of wheat. A quantity of wheat heads at about 43 per cent moisture and in the early dough stage of development was harvested June 8, 1949. About 50 g were placed in each of six different Erlenmeyer flasks. Two of the flasks received no further treatment and served as controls. They were placed in the respirometer immediately. The second set of two were flushed with an excess of ethylene which had been diluted 500 times with air. These bottles were then sealed and stored at room temperature for 24 hours and then placed in the respirometer.

The third set of two flasks was flushed with an excess of pure ethylene and immediately placed in the respirometer. Air



passing into these containers was first bubbled through water that had been saturated with ethylene.

At the end of six days one member of each set was removed from the respirometer and examined. All the flasks contained abundant free moisture. Some of the wheat heads were completely covered with mold while others from the same flask appeared to be almost free of mold. There was no essential difference in the appearance of the different samples. These samples were then discarded without further testing.

On the tenth day the remaining samples were removed from the flasks and examined. Droplets of water covered the sides of the flask and the wheat heads were covered with mold. Moisture content was determined by the two-stage oven method. The samples were then dried, ground and tested for free fat acidity. Respiration and other data obtained in this experiment are presented in Table 3 and Fig. 1.

Reference to Table 3 and Fig. 1 indicate that all of the samples respired at an extremely high rate. This was undoubtedly due, at least in part, to the very high moisture content of the samples.

The samples treated with ethylene exhibited higher maximum rates of respiration than the untreated sample. This increase is comparatively small, and in view of the heterogeneous nature of the samples, it can not be considered significant. Therefore, it can be said that ethylene apparently has no effect on the respiration of fresh immature whole heads of wheat.



Table 3. Influence of ethylene on fresh wheat seeds in dough stage. (About 50 g wheat seeds harvested June 8. Germination, regular 19%, prechilled 47%.)

Sample number	Treatment	Respiration mg CO <sub>2</sub> /100 g dry matter	Fat	Acidity	Two-stage moisture
		Initial	Total	after test	after test
		daily rate	for trial		per cent
Original sample		--	--	--	47.7
I	None	1491	11,720	38.0	55.5
II	None	1479	--	--	--
III	stored in atmosphere of dilute ethylene for 24 hours	1650	13,490	50.9	58.3
IV	Same as III	1718	--	--	--
V	Excess pure ethylene	1655	11,970	35.3	53.0
VI	Excess pure ethylene	1739	--	--	--

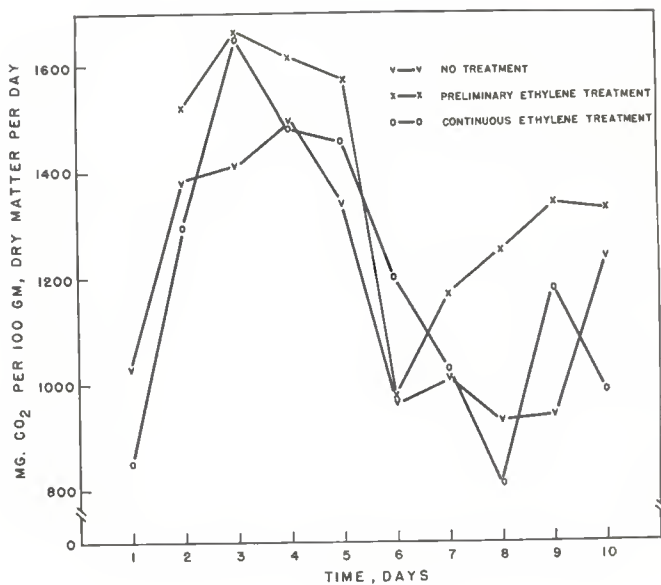


Fig. 1. Influence of ethylene on fresh wheat heads in dough stage.

The Effect of Ethylene on Immature Threshed Wheat. The purpose of this experiment was to observe the effects of ethylene on respiration and associated changes in immature threshed wheat.

The first sample of immature grain for this experiment was harvested by hand on June 14, 1940, when the grain had a moisture content of 43 per cent. The kernels were removed from the heads by hand threshing. Those grains which were submitted for germination tests had been dried at about 41° C. for two days and this undoubtedly accounts for the reported low values of 24 per cent by the regular method and 32 per cent by the perched method.

About 50 g of the damp grains were placed in each of three Erlenmeyer flasks. One of the samples was placed in the respirometer immediately without further treatment and acted as a control. The second sample was flushed with an excess of ethylene diluted 1:500 with air, then it was sealed and stored for 24 hours at room temperature before being placed in the respirometer. The third sample was flushed with an excess of pure ethylene and placed in the respirometer at once. Air passing into the third bottle was first bubbled through water that had been saturated with ethylene.

Because of the very high respiration rate of this set of samples, the flasks were removed from the respirometer only four days after treatment. The kernels were found to be matted together with green and white mold and gave off a strong acetic or ester-like odor. A portion of each sample was ground and

tested for free fat acidity and a two-stage moisture determination was run on the remainder. The data obtained are given in Table 4.

Reference to Table 4 indicates that there was no significant respiration change resulting from treatment with ethylene. There was a considerable difference in the free fat acidity, with an increase being observed in connection with increasing concentrations of ethylene.

When the wheat in the field had reached a moisture content of 80.2 per cent, another sample was harvested and then threshed on a small mechanical thresher. Rather drastic treatment was necessary to separate the chaff from the grain, and this resulted in a fairly high percentage of damaged kernels. This undoubtedly accounts for the relatively low values reported for germination of the air-dried grain. The germination was 57 per cent by the regular method and 82 per cent by the prechilled method.

It was thought advisable to use a mold inhibitor with some of these samples in order to observe, if possible, the respiration of the wheat uncomplicated by the additional carbon dioxide production of the proliferating molds. To this end, thiocarbamide was introduced into some of the samples. This chemical had been found to depress considerably mold growth on moist wheat without appreciably affecting the germination.

Five 100-g samples of this wheat were weighed into Winkler flasks on the same day that it was harvested. The first flask was placed in the respirometer at once without further

Table 4. Effect of ethylene on immature wheat grains.

Sample number	Treatment	Respiration mg CO <sub>2</sub> /100 g dry matter	Maximal daily rate attained	Total after test trial	Two-stage acidity: moisture after test per cent
Original wheat	--	--	--	--	43.0
I	None	4,566	12,800	90.2	47.1
II	Stored in dilute ethylene atmosphere for 24 hours	5,214	11,140	110.0	46.5
III	Excess pure ethylene	4,943	--	150.6	47.6

(Grain harvested June 14, 1940; germination regular 94%;  
prechilled 32%; trial run 4 days.)



treatment and acted as a control. One gram of finely powdered thiocarbamide was added to the second flask, the contents were mixed thoroughly, and the flask was placed in the respirometer. One gram of thiocarbamide was added to the third flask, which was then flushed with an excess of a 1:500 ethylene in air mixture and set aside for one day.

Sample IV was placed into the respirometer at once without further treatment. After five days it was removed from the respirometer, flushed with ethylene in air; 1:500 mixture, set aside for one day, and then replaced in the respirometer.

Sample V was flushed with an excess of undiluted ethylene and then placed in the respirometer. Air passing into this sample was first bubbled through water saturated with ethylene.

After 10 days, samples were removed from the respirometer and examined. The two-stage moisture values were determined, and then the remainder of the grain was air-dried. Dry kernels were submitted for germination and the remainder ground and tested for free fat acidity. No difference in the gross appearance of the various samples was noted. Data of this experiment are presented in Table 5.

Reference to Table 5 indicates that a decrease in respiration and free fat acidity and an increase in germination resulted from the use of thiocarbamide. Thus the efficacy of this substance as a mold inhibitor for damp wheat is again confirmed.

In the control sample, and in all samples treated with ethylene there was an increase in the germination as determined

Table 5. Effect of ethylene treatment on immature wheat. (100 g of wheat "C"; trial conducted for 10 days.)

Sample number	Treatment	Respiration mg : CO <sub>2</sub> /100 g dry matter	Two-stage : moisture	Germination : after trial	Acidity : after trial
		Maximum : daily ratio : for trial	after trial	after trial	after trial
		integrated : trial : per cent	per cent	per cent	per cent
Original wheat	--	--	25.3	57	82
I None		30.7	18.2	62	60
II 1.0 g thiocarbamide		61.4	21.0	74	93
III 1.0 g thiocarbamide stored under dilute ethylene for 24 hrs.		62.1	21.4	75	75
IV stored under dilute ethylene for 24 hrs. after 5 days in respirometer		115.0	21.5	60	75
V excess pure ethylene		64.5	32.1	64	75

85.9

61.7

by the regular method and a decrease in the germination as determined by the prechilled methods in comparison with the values obtained for the original wheat. This observation cannot be accounted for by the operation of any known factor. Possibly the mold growth stimulated some of the embryos with a resultant effect similar to prechilling and killed some of the less resistant embryos.

Both the thiocarbamide-treated samples showed practically the same respiration rates, while the ethylene-treated samples without thiocarbamide showed fairly high rates. Possibly the ethylene stimulates mold growth slightly but does not stimulate the respiration due to the seed metabolism, although it is more likely that the varying respiration rates were only a reflection of the different moisture contents of the samples.

The Effect of Ethylene Treatment on Mature Wheat. On June 24, 1940, when the moisture of the wheat in the experimental plot had been estimated to be approximately 14 per cent, several bushels were harvested by a small combine. This is the sample "D" mentioned previously.

A quantity of this wheat was placed in a 33 liter drum. Thirty-three ml of pure ethylene were run into the drum which was then hermetically sealed. The grain was shaken thoroughly. This provided a dilution of ethylene with air in the ratio of 1:1000.

A quantity of untreated wheat from the original sample was brought to about 19 per cent moisture by the addition of dis-

tilled water one day after harvesting. About 24 hours later enough pure ethylene was introduced into the container to make a 1:1000 mixture with the air present. This wheat was stored at 50° F. for two days before being placed in the respirometer.

A quantity of both the unwetted ethylene-treated wheat and the dry untreated wheat were brought to 19 per cent moisture by the addition of distilled water. These samples were stored at 50° F. overnight.

On June 23, 1949, 300 g each of the dry untreated wheat and the dry ethylene-treated wheat were placed in the respirometer. One hundred-gram samples of the wetted untreated wheat, the wetted ethylene-treated wheat and the ethylene-treated wetted wheat were placed in the respirometer at the same time.

On the tenth day the two dry wheat samples were removed from the respirometer, examined and submitted to various tests. After a total of 14 days the remaining samples were treated similarly.

Data of this experiment are presented in Fig. 2 and Table 6. The respiration rates of the wheat samples at 14 per cent moisture are not shown on the graph because of the insignificant amount of carbon dioxide respired.

Reference to Table 6 and Fig. 2 indicates that a greatly increased respiration rate resulted from an increase of a few per cent in the moisture content and that this increase was accompanied by a lowered germination, a higher free fat acidity value, and an increase in the moisture content.

Table 6. Effect of ethylene on mature wheat. (Plant "D", 12 days trial.)

Sample number:	Treatment:	Respiration mg C <sub>2</sub> H <sub>4</sub> /100 g dry matter	Maxima	Total	Daily rate for attained	Before trial	After trial	Before trial	After trial	Before trial	After trial
Grams						mg	mg	mg	mg	mg	mg
I	300 None	5.51	35.3	91	91	--	95	14.2	13.1	57.7	
II	300 Ethylene-air, 1:1000	6.79	30.7	88	88	--	98	14.0	14.1	63.7	
III	100 Conditioned to high moisture	151.0	1345.0	83	91	0	0	19.3	20.0	93.0	
IV	100 Treated with ethylene then dampened	193.0	1611.0	83	93	0	0	19.6	20.4	103.0	
V	100 First dampened, then treated with ethylene, 1:1000	164.0	1557.0	84	83	0	0	19.7	20.5	99.6	



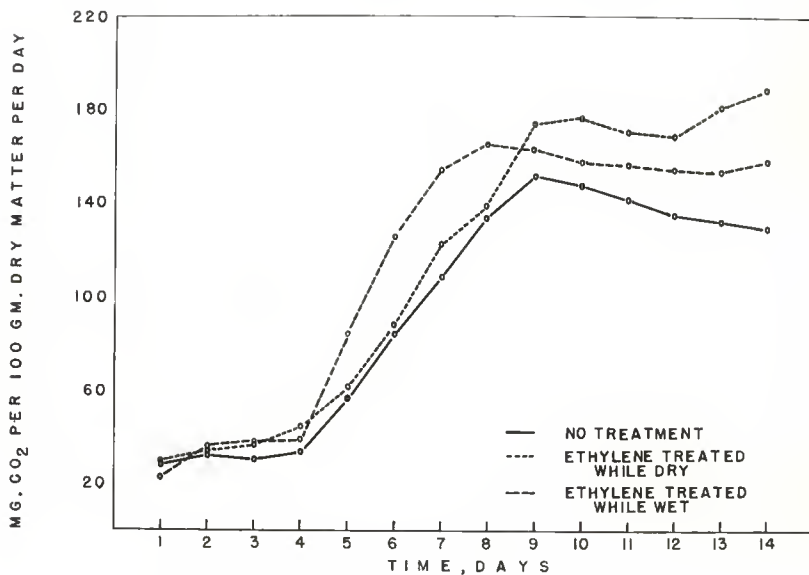


Fig. 2. The effect of ethylene on mature wheat.

No effect attributable to ethylene treatment was observed.

The Effect of Ethylene on Dried and Revetted Wheat. The purpose of this experiment was to study the effect of ethylene on dry mature wheat and on dried immature wheat which had been conditioned to a fairly high moisture content after being stored for some time at a low moisture content.

Immature wheat "C" which had been dried to about 13.5 per cent moisture by exposing it in a thin layer to the air, and which had been stored for several weeks at the lower moisture content and at room temperature was conditioned to about 25 per cent moisture. The conditioned wheat was stored at room temperature for two days before it was placed in the respirometer. This sample will be designated "CLST" in this discussion.

Part of the above-mentioned conditioned wheat was treated with a mixture of ethylene in air 1:500 for 72 hours. This sample will be designated "CLSTE" in the discussion.

Some of the wheat sample "C" had been dried to about 12 per cent moisture soon after harvesting and then treated with ethylene in air, 1:1000 mixture, for several days. A sample of this wheat was now conditioned to about 25 per cent moisture and stored at 50° F. for three days. This sample will be designated "CBLT".

One hundred-gram portions of these samples were placed in the respirometer and the respiration rates were observed for 15 days. The results of this experiment are given in Fig. 3 and Table 7.

Table 7. Effect of ethylene on dried and rewetted immature wheat. (100 gram samples "C" wheat; trial conducted for 15 days.)

Sample designation:	Treatment	Respiration mg :		
		CO <sub>2</sub> /100 g dry	matter	Two-stage moisture
		Maximum	Total	after
		daily rate	for	trial
		attained	trial	per cent
C13T	Immature wheat dried to about 12% moisture, then conditioned to 25% moisture	854.0	8474.0	25.0
C13TB	Same as above, treated with 1:500 ethylene after moistening	880.0	6906.0	24.8
C13TT	Immature wheat dried, then treated with ethylene, then conditioned to 25% moisture	417.0	2615.0	23.5

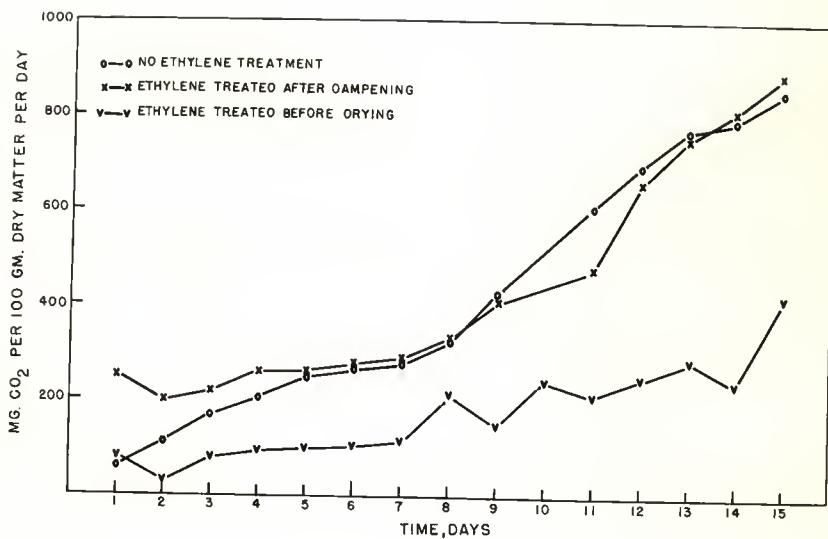


Fig. 3. Effect of ethylene on dried and rewetted immature wheat.

The results of this experiment indicate that the biological changes which wheat undergoes when it is dried and then dampened do not affect its lack of sensitivity to ethylene. No differences were observed in the respiration rates which could not be attributed to factors other than ethylene treatment. It is believed that the difference in moisture content is sufficient to account for the relatively low respiration observed for wheat "CLLET" since the other ethylene-treated sample had a respiration rate practically identical to that of the untreated wheat.

It was also deemed advisable to study the effect of ethylene on mature wheat which had been harvested at a normally low moisture content and then conditioned to a higher moisture content after some weeks in storage. "D" wheat was used in this experiment, which was essentially a repetition of Part IV of this series.

Twenty ml of water were added to 300 g of "D" wheat to bring it to about 20 per cent moisture. This was stored at 50° F. for two days, and then 100 g were placed in the respirometer. The mark "D22" identifies this sample.

After the "D" wheat which had been brought to 20 per cent moisture had been stored for one day at 50° F., 100 g were removed and treated with five cc of pure ethylene gas. This wheat was then stored for one more day at 50° F. before being placed in the respirometer. This sample is to be designated "D2E2".

Six and three-tenths ml of distilled water were added to



a 95.7 g sample of the "D" wheat which had been treated with ethylene while it was still dry -- soon after harvest. This sample was then stored for one day at 50° F. before being placed in the respirometer. The designation "DWTG" identifies this sample.

These samples were left in the respirometer for 15 days. The results of this experiment are given in Table 3 and Fig. 4.

Reference to Table 3 and Fig. 4 indicates that the wheat which was treated with ethylene after it was moistened had a much higher maximum rate of respiration and that its respiration rate decreased much more slowly after the peak had been reached. It is difficult to ascribe this effect to any factor besides the ethylene treatment since the moisture differences here were quite small. However, corresponding differences were not observed for the samples studied in Part III of this series of experiments. In view of this apparent contradiction, no definite conclusions can be drawn.

The Effect of Ethylene on Newly Milled Flour. This experiment was carried out for the purpose of observing any improving effect which ethylene might have on flour milled from newly harvested wheat.

A sample of the "C" wheat previously described was dried, stored for a few weeks, then tempered and milled into flour. No ethylene treatment was applied due to the limited sample available. This flour was used in this experiment in order that some idea of the effect of the naturally occurring maturation

Table 8. Effect of ethylene on dried and rewetted wheat.  
(100 grams; trial conducted for 15 days.)

		Respiration mg		
		CO <sub>2</sub> /100 g dry	Two-stage	
Sample	Treatment	matter	moisture	
designation:		maximum	Total	
		daily rate: for	after	
		attained	trial	per cent
DT2	Conditioned to about 20% moisture	142	1246	18.22
DTM2	Conditioned to about 20% moisture, then treated with ethylene	236	2437	18.99
DET2	Treated with ethylene while dry, then conditioned to about 20% moisture	190	1703	18.33

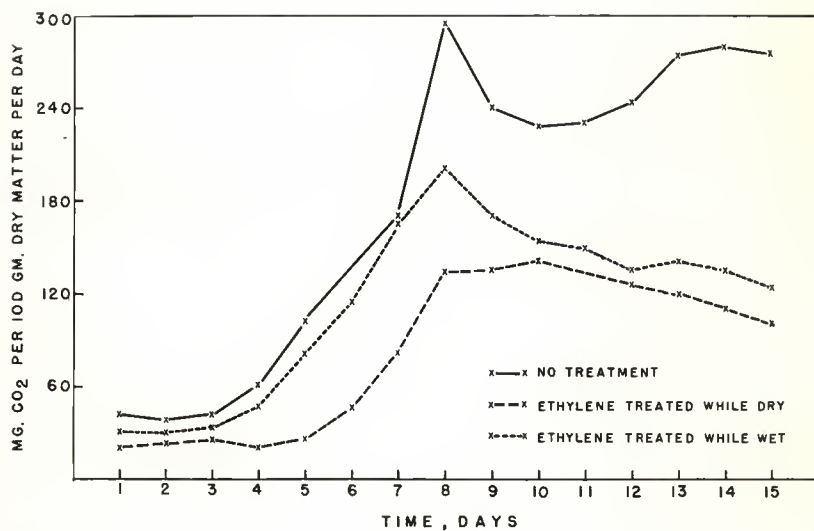


Fig. 4. Effect of ethylene on dried and rewetted wheat.

of the wheat on the resultant flour could be obtained by comparing it with the flour described below.

A sample of "D" wheat was tempered and milled. A two kg sample of this flour was placed in a small experimental bleacher and agitated for several minutes with two liters of pure ethylene. Another sample of the same flour was treated in the same way with several liters of a mixture of ethylene diluted with 100 parts of air.

Pound loaves were baked from each of the above samples of flour. In addition loaves were baked from the flour milled from "D" wheat plus potassium bromate in order to observe the flour's response to oxidation.

The following formula was used for the bread:

700 g flour  
35 g sugar  
14 g salt  
21 g shortening  
14 g yeast  
Absorption, 57 per cent

The weight and volume of the loaves were determined and they were scored for break and shred, grain, color and texture. Results of this experiment are given in Table 9.

Reference to Table 9 indicates that the flour milled from wheat harvested at 25.3 per cent moisture gave loaves with better volume than flour from grain harvested a week later. Potassium bromate improved the volume and texture of the loaves

Table 9. Effect of ethylene on newly milled flour.

Wheat moisture at date at harvest: 1949	Date of harvest: 1949	Flour extraction: per cent	Flour treatment: per cent	Volume: cc	Leaf characteristics color: green and yellow	Texture: score	Color: green and yellow	Texture: score
23.2	June 10	63	None	2050	510	35	Creamy yellow	Poor
14.2	June 24	63	None	2000	523	35	Creamy yellow	Poor
14.2	June 24	63	Dilute ethylene	2010	523	35	Creamy yellow	Poor
14.2	June 24	63	Pure ethylene	2050	526	35	Creamy yellow	Poor
14.2	June 24	63	.002% K <sub>2</sub> O <sub>3</sub>	2225	---	35	Creamy yellow	Poor

Other characteristics of the flour milled from the wheat harvested June 24, 1940, at 14.2% moisture:

Farinograph absorption value: 52%  
 Protein (14% moisture basis): 8.5%  
 Ash: 0.42%  
 Maltose value: 204  
 Maximum amylograph viscosity: 985



to which it was added. Ethylene apparently had no effect whatsoever on the loaf characteristics.

#### Influence of Carbon Tetrachloride on Respiration and Germination

Inasmuch as carbon tetrachloride is a widely used constituent of wheat fumigants, and in view of the results obtained by Lammour et al. in the previously mentioned investigations, it was deemed to be advisable to study the influence of this chemical on the respiration and germination of wheat of high moisture content.

Six 300-g samples of wheat "A", conditioned to 19.2 per cent moisture were placed in 500 ml Erlenmeyer flasks. Various amounts of carbon tetrachloride were added to the flasks and the wheat and chemical were thoroughly mixed. The flasks were immediately placed in the respirometer and aeration begun. One sample was provided with continuous carbon tetrachloride vapor treatment by passing the incoming air through liquid carbon tetrachloride. Tests showed that, under the conditions used in these experiments, about 2 g of carbon tetrachloride vapor were applied to the grain each day.

This respirometer trial was carried out for 10 days. Results of this experiment are presented in Table 10 and Fig. 5.

Reference to Table 10 and Fig. 5 indicates that, under ordinary conditions, carbon tetrachloride is an effective fungistatic agent for damp wheat only if comparatively large

Table 10. Inhibition of respiration by carbon tetrachloride (300 g of wheat "A" in each sample; trial conducted for 10 days).

Sample number	Moisture after trial per cent	Treatment CCl <sub>4</sub> ml	Respiration mg			Germination after trial per cent
			CO <sub>2</sub> /100 g dry matter	maximum daily rate	total for trial	
Original wheat	19.2	--	--	--	--	79
I	17.7	None	67.4	426.0		6
II	17.7	0.1	71.1	424.0		7
III	17.5	0.3	67.0	359.0		15
IV	17.3	0.6	74.6	330.0		23
V	17.7	1.0	67.3	245.0		44
VI	17.4	Continuous vapor	16.0	117.0		57

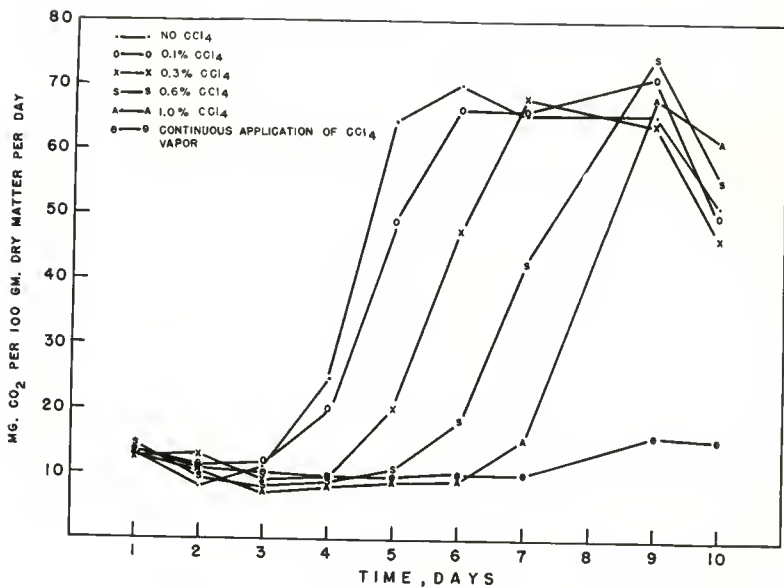


Fig. 5. Inhibition of respiration by carbon tetrachloride.

amounts are used. A significant decrease in germination occurred in all samples of wheat, even in those which did not respire rapidly, indicating that a certain loss in value of seed wheat may occur if it is treated with this chemical. The work of Passerini (1932) is of interest in this connection. He found that a short immersion of dry wheat in carbon tetrachloride accelerates germination and that wheat kept immersed in carbon tetrachloride for 10 months germinated four per cent.

The experiment was repeated using the same procedure with "B" wheat samples. This repetition was thought to be advisable because of the unknown composition and variety of the wheat previously used and because of its low germination percentage. Exactly the same procedure was followed as in the other experiment except that the trial was continued for 14 days. Results are given in Table 11.

Reference to Table 11 indicates that respiration figures somewhat analogous to those obtained in the previous experiment were again observed. However, minor but significant differences were apparent. The maximum respiration attained by the COT wheat was less than one-half that of the other sample. This could be accounted for by the difference in soundness of the two wheats. Another point of difference was the decreasing respiration which was observed during several days at the beginning of the trial in those samples treated with fixed dosages of carbon tetrachloride. This may indicate a depressive effect on the respiration of the seed itself, such as was ob-

Table 11. Inhibition of respiration by carbon tetrachloride (300 g of wheat "W" in each sample; trial conducted for 14 days).

Sample number	Moisture : after trial : per cent :	Treatment : $\text{CCl}_4$ ml :	Respiration mg : $\text{CO}_2/100$ g dry matter :	Maxima : daily rate : attained :	Total : for trial :	Germination after trial : per cent :
Original wheat	16.3	--	--	--	--	36
I	17.2	None	23.2	243.0		43
II	17.0	0.1	27.2	332.0		53
III	16.4	0.3	25.2	191.0		53
IV	17.9	0.6	31.1	217.0		63
V	16.0	1.0	23.3	152.0		62
VI	17.5	Continuous vapor	11.9	146.0		92



served by Larmour et al. A major point of difference between the results of the two experiments is the higher respiration observed in this trial with continuous carbon tetrachloride treatment than with treatment with fixed dosages.

Not much damage to wheat germination by carbon tetrachloride treatment is indicated by the results of this experiment. The loss in germination was only four per cent for that sample under the continuous vapor treatment, and a much larger loss attributable to side effects of the mold growth may be noted in the control sample and in the samples treated with the smaller amounts of carbon tetrachloride.

The third experiment was instituted to determine the effect of carbon tetrachloride on the germination and respiration of wheat of various moisture contents. "B" wheat samples of various weights and of various moisture contents were used in this experiment. The samples at 19 per cent and at 23 per cent moisture were provided with continuous carbon tetrachloride vapor treatment, while samples at 11 per cent and at 13 per cent moisture were observed both with and without vapor treatment. The data of this experiment appear in Table 12 and Fig. 6.

Reference to Table 12 and Fig. 6 indicates that carbon tetrachloride kept the respiration values within safe limits and had little deleterious effect on the germination. Respiration values obtained for the wheat at 11 per cent and at 13 per cent moisture were very low and it was not considered de-

Table 12. Inhibition of respiration by carbon tetrachloride ("CCl<sub>4</sub>" wheat; trial conducted for 12 days).

sample number	Two stage : moisture : before : trial : for cont	moisture : after : trial	CCl <sub>4</sub> : treatment	respiration mg : CO <sub>2</sub> /100 g dry matter : treatment : daily rate : attained : trial	CO <sub>2</sub> /100 g dry matter : total : treatment : for iper cent	Correlation : after : treatment
I	11	12.8	Continuous vapor	0.10	0.70	93
II	11	11.3	None	0.00	0.00	97
III	13	13.0	Continuous vapor	0.40	2.73	92
IV	13	13.1	None	0.23	1.13	94
V	19	12.2	Continuous vapor	26.1	271.0	95
VI	23	24.0	Continuous vapor	146.0	1207.0	92

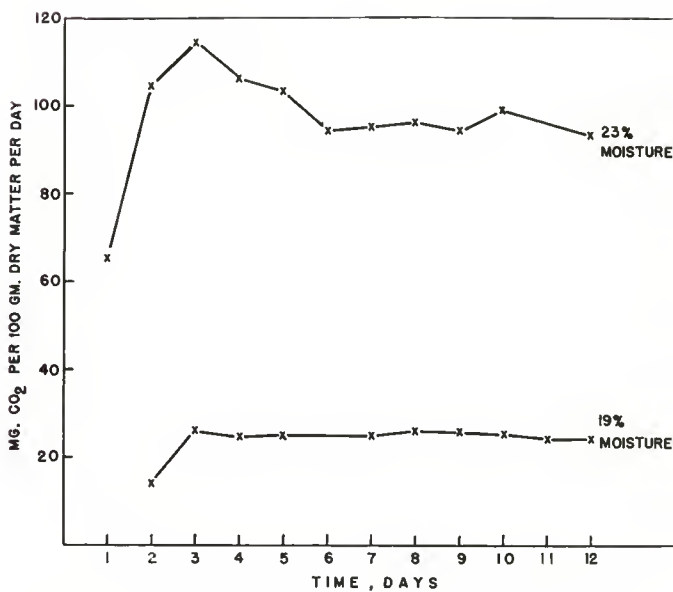


Fig. 6. Inhibition of respiration by carbon tetrachloride.

sirable to include them in the graph.

At this point, it seemed advisable to conduct another experiment to extend the number of moisture percentages for which respiration values were available and to confirm the results obtained in the preceding experiment. To this end, three sets of two samples each of "B" wheat conditioned to various moisture contents were placed in the respirometer. One member of each set was ventilated and the other member was aerated with air containing carbon tetrachloride vapor. The first of these sets consisted of wheat at about 19 per cent moisture; the second of wheat at 21.5 per cent moisture, and the third of wheat at 24 per cent moisture.

At the end of 10 days, the samples were removed from the respirometer and tested for moisture by the two-stage oven method. Portions of each sample were submitted for germination and tested for free fat acidity. Results of this experiment are given in Table 13 and Fig. 7.

Reference to Table 13 and Fig. 7 indicates that application of carbon tetrachloride caused a decrease in respiration and an inhibition in the development of free fat acidity. The respiration values obtained were of the same order as those observed in the previous experiment.

From the results of these experiments with carbon tetrachloride, it seems likely that the chemical would have beneficial results when applied to wheat which must be stored at moisture contents which are conducive to mold growth. A slight

Table 13. Inhibition of respiration by carbon tetrachloride ("B" wheat; trial conducted for 10 days).

sample number	two stages : moisture : before : after : trial : trial : per cent	CCl <sub>4</sub> : treatment	respiration mg : CO <sub>2</sub> /100 g dry matter : maximum : total : daily rate: for : attained : trial :	fat : acidity : after : trial
I	10 18.2	Continuous vapor	21.0 153	19.4
II	19 17.9	None	39.9 217	20.9
III	21.5 19.7	Continuous vapor	57.4 351	17.5
IV	21.5 19.3	None	69.5 527	26.5
V	24 21.3	Continuous vapor	76.1 716	18.2
VI	24 22.0	None	166. 939	30.5



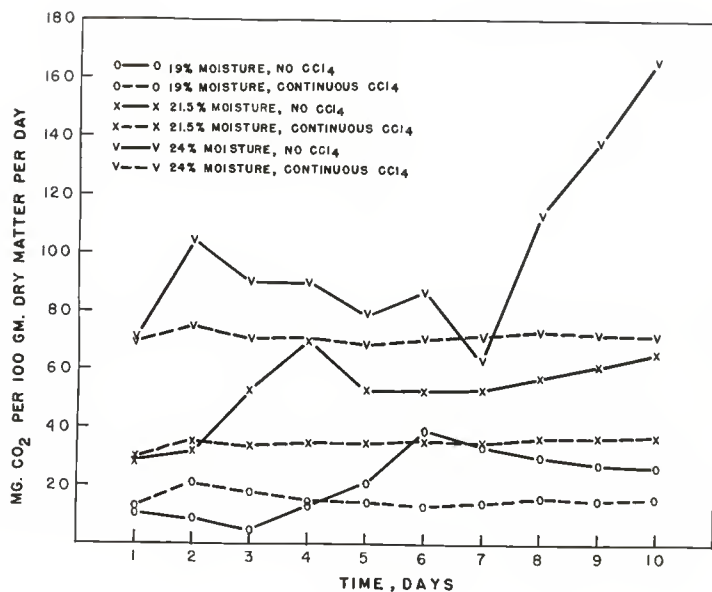


Fig. 7. Inhibition of respiration by carbon tetrachloride.

decrease in germination could be expected but this effect would undoubtedly be offset by the inhibition of mold growth and the concomitant deleterious changes in the grain. The decrease in baking quality of damp wheat treated with carbon tetrachloride which was noted by Larmour and Bergsteinsson will need to be verified before recommendations for commercial practice can be made.

### Tests of Possible Grain Preservatives

Screening Tests for Mold Inhibitors. The purpose of this experiment was to observe the relative effectiveness of various chemical compounds in retarding mold growth and related deterioration in samples of grain at high moisture.

Fifty-gram samples of clean GWT wheat at 80 per cent moisture were placed in small screw-capped bottles. On these samples was placed 0.1 cc of the liquid compounds to be tested or 0.1 g of the solid compounds. The bottles were then sealed and the contents mixed. They were stored at room temperature and occasionally examined for visual evidences of mold growth. After 14 days the bottles were opened and the contents spread out on tin can lids for drying. When sufficiently dry, samples of the grain were submitted for germination tests and free fatty acid determinations were run on the remainder.

Results of this experiment are given in Table 14. The ratings are based on a careful evaluation of all the factors

Table 14. Effect of chemical treatment on seed germination and development of mold and fat acidity in damp stored wheat. (Original germination of wheat, 97%; fat acidity 23.3.)

Test no.	Treatment	Manufacturer	First Period			Disqualifying factors			Rating
			Control	No. treated	Days	Germination (%)	Acidity (%)	Factor	
0	Control	--	4	27	74.2				
1	Propylene oxide	Carbide & Carbon	-	0	34.4				A
2	Alkaterge C	Commercial solvents	4	17	111.43				C
3	Denyltrimethyl- ammonium chloride	"	4	15	69.0			Toxic	B
4	Butyl borate	"	-	0	40.5			Toxic	C
5	Tris(hydroxy- methyl)-nitro- methane	"	4	21	53.5			?	B
6	2 nitro, 2 methyl, 1-3, propanediol	"	8	25	69.0			?	B
7	Hydroxylammonium chloride	"	-	0	60.1			Toxic	C
8	2 amino, 3 methyl, 1,3 propanediol	"	-	77	55.0			?	A
9	Thiocarbamide	Merck	-	94	59.4			Undesirable odor	A
10	Diethyl oxalate	Commercial solvents	-	0	53.7				B
11	n, n dicarboxy- ethyl benzene- sulfonamide	Pyandotte	-	27	252.53			?	C
12	Methylbenzene	"	4	19	53.0				B
13	n, n dicarboxethyl benzenesulfonamide	"	4	20	49.4			?	B



Table 14 (cont.).

Test no.:	Treatment:	Manufacturer:	First : old :	Second : old :	Disqualifying : factor : : (days) :	Disqualifying : factor : : (days) :	Disqualifying : factor : : (days) :
Second Series							
32	Control. No treatment	--	5	12	07.0	-	-
33	2, 3 dichloro 1, 4 naphthoquinone	Naugatuck	-	61	48.5	?	A
34	2 methyl 1, 4 naphthoquinone	"	-	0	30.2	?	A
35	Triethylene Glycol diacetate	Kessler	7	12	42.9	?	B
36	Thioacetic acid	Arepahee	-	0	152.03/	Isachrymator	C
37	Dimethyl di-chlorosuccinate	"	-	0	99.03/	?	C
38	Propylene chlorohydrin	Carbide & Carbon	-	0	39.9	?	A
39	Parahydroxy-benzoic acid	Neyden	6	10	172.03/		C
40	Methyl parasept	"	12	96	61.5		B
41	Ethyl parasept	"	9	55	43.7		B
42	Propyl parasept	"	9	14	64.3		B
43	Butyl parasept	"	6	13	67.3		B
44	Tenail	"	-	9	70.33/		B
45	Anilic acid	"	6	16	61.2		B
46	Para-amino-benzoic acid	Eastman	6	13	56.1		B
47	Propylene Glycol Ether oxide	"	9	12	79.33/		B
Carbide & Carbon							Toxic, flammable, Volatile.



Table 14 (cont.).

Test no.	Treatment	Manufacturer	First : : mold : : (days) :	Food : : germi- : : visible : : (days) :	at : : acid : : 5 : : per MOL :	Disqualifying : : factor : : rating :	Test- : tive : : rating :
43	Methyl oxide	Shell	-	0	30.2	Flammable, Toxic.	C
49	Lauroic acid	Armour	12	64	47.1		A
50	Palmitic acid	"	6	15	67.7		B
51	Stearic acid	"	6	13	68.7		B
52	Arquad 89	"	5	54	73.0		A
53	Arquad 2-65	"	-	73	44.1		A
54	Neofat 3-R	"	5	9	45.0		B
55	Capric acid	"	-	6	64.7	Flammable, Toxic.	C
56	di-tert-butyl peroxide	Shell	5	9	77.0	Flammable, Toxic.	C
57	di-isopropylamine	"	12	13	71.7	Flammable, Toxic.	B
58	Ethylene chlorohydrin	Eastman	-	0	23.1	Toxic.	C
59	1,3 dichloropropene 1	Shell	-	0	33.9	?	A
60	Diethyl sulfolane	"	5	31	46.4	Toxic	C
61	Methylal alcohol	"	6	0	33.3	Flammable, Toxic, lachrymator.	C
62	Methylal chloride	"	12	0	60.3	Flammable, Toxic.	C
63	Acrolein	"	-	0	47.5	Flammable, lachrymator, volatile.	C
64	Allyl alcohol	"	-	0	45.4	Flammable, Toxic.	C

Table 14 (cont.).

Test no.	Treatment	Manufacturer	First mold visible (days)	Food	Test	Disqualifying factor	Result
65	Allyl chloride	Shell	15	0	30.0	Flammable, Toxic.	C
66	Epichlorohydrin	"	-	0	33.9	Flammable, Toxic, Lachrymator.	C
67	Methylene glycol	"	13	20	71.7		D
68	1,2,3 trichloropropane	"	-	57	47.1	Toxic	A
69	beta-dithiocarbonylpropionic acid	E. F. Goodrich	6	17	30.03	?	C
70	beta-carboxymethyl benzothiazyl S-sulfide	"	5	14	39.4	?	D
71	beta-isothionureido propionic acid	"	6	15	46.2	?	A
72	Rhodamine	"	6	31	46.2	?	A
73	beta-propiolactone	"	-	3	24.6	Vesicant, Toxic.	C
<u>Thiyl Series</u>							
Control. No.	-	-	3	27	52.2		
74	Treatment. Propylene glycol diacetate	Cosler	12	3	24.6	?	A
75	Dipropylene glycol diacetate	"	3	9	32.6	?	D
76	Resin-1	"	-	13	52.3	?	D
77	Anthranilic acid	Merck	3	30	34.6		A
78	Tripropionin	"	-	0	24.3	?	A

Table 14 (cont.).

Test no.:	Treatment	Manufacturer	Patent no.:	Food :	Patent no.:	Gen. acid :	Pat. no.:	Disqual. factor :	Rating :
:	:	:	:	:(days):	:(days):	:(days):	:(days):	:(days):	:(days):
79	E-acetyl anthranilic acid	Rock	3	20	66.13/	?			B
80	E-acetyl ethylanthranilate	"	3	13	37.1	?			B
81	Methyl n amino benzoate	"	3	33	52.0				B
82	Ethyl p amino benzoate	"	6	31	50.3				A
83	E-acetyl methyl p aminobenzoate	"	6	40	43.4				B
84	n butyl vanillate	"	3	13	62.23/	?			B
85	E-propionyl methyl anthranilate	"	3	45	30.2	?			A
86	n benzoyl methyl anthranilate	"	3	19	53.9	?			B
87	Ethyl anthranilate	"	6	47	26.9	?			A
88	Propylene dipropionate	"	-	0	30.3	?			A
89	Ethylene di-propionate	"	3	8	53.4	?			B
90	Ethyl anthranilate	"	6	44	52.7				A
91	Ethylene chloride	Dupont	0	20	20.6				A
92	Trichlorethylene	"	-	69	23.0				A
93	Tetrachlorethylene	"	-	19	34.1				B
94	Chloroform	"	14	13	33.7				B
95	Pentachloroethane	"	-	17	24.1				A
96	Tetrachloroethene	"	-	0	20.0				A

Toxic  
Toxic  
Toxic

Table 14 (concl.).

Test no.:	Treatment	Manufacturer	First : :mold : :visible : :(Date) :	Seed : :Germi- : :ation : : %	Disqualifying : :factorial : : %	Rating :
<u>Fourth Series</u>						
Control. No	---		3	44	30.2	-
97 Pluracol, P762	---	Yandotte	14	62	26.3	A
98 Lot O-2776 Pluracol, P234,	"		3	34	60.33/	C
99 Lot O-3025 Pluracol,	"		6	43	52.33/	B
100 Lot O-3037 Pluracol, P762,	"		3	31	30.1	B
101 Lot O-3113 Pluracol,	"		5	41	34.7	B
102 Lot O-3224 Pluracol,	"		5	39	37.3	B
103 Lot O-3379 Pluracol, P2156,	"		3	38	36.6	B
104 Lot O-376 Pluracol, P1270,	"		3	29	37.0	B
Lot O-3371						

1/ Absence of the word "toxic" indicates that the toxicity of the substance is not thought to be sufficiently great to cause hazards to persons contacting the amounts commonly encountered in normal procedures. Presence of a question mark indicates that satisfactory evidence concerning non-toxicity was not available.

2/ In the rating column, A indicates that the effectiveness of the compound is sufficiently promising to indicate the desirability of further tests, B indicates that

the compound is apparently without significant preservative effect, C indicates that the compound should be eliminated from further consideration because of deleterious effects on the grain, or because of toxicity, flammability, odors, etc.

3/ Chemical probably contributed to acidity.

"Parasept" is a trade name of Heyden Chemical Co. for salt of para-hydroxybenzoic acid.

4/ Arquad S is a trade name of Armour Co. for soy trimethyl ammonium chloride.

5/ Arquad 2-C is a trade name of Armour Co. for di-coco dimethyl ammonium chloride, 75% active.

6/ Neofat S-R is a trade name of Armour Co. for a mixture of linoleic and oleic acids.

7/ Pluracol is the trade name of Yandotte Chemical Co. for various polymers of propylene oxide.



which are listed in the table. The significance of the ratings is given in the footnotes. If a compound left a toxic residue or if it was sufficiently toxic to be hazardous to health under normal handling procedures, it was given a "C" rating. In some cases, a foul odor or extreme flammability contributed toward a "C" rating for a compound. Free fat acidity values were interpreted as follows: compounds which appreciably increased the free fat acidity value of treated wheat above that of the control were given "C" ratings; compounds which had no appreciable effect on the development of free fat acidity in the treated wheat were given a "B" rating; compounds which considerably retarded the development of free fat acidity in treated wheat were given a rating of "A" if other characteristics of the compound did not disqualify it from further consideration.

Effect of Mold Inhibitors on the Respiration of Moist Wheat. In order to elucidate clearly the effect of some of the more effective mold inhibitors on damp grain, it was decided to measure the effect which they had on respiration. The conditioned wheat was placed in Erlenmeyer flasks and treated with the chemical if the chemical was a liquid or a solid, and if the chemical was a gas, the conditioned wheat was treated in a small experimental bleacher prior to being placed in the respirometer. Three different trials were conducted.

The results of this experiment are presented in Tables 15, 16 and 17 and Figs. 8 and 9.

Reference to Table 15 and Fig. 8 indicate that propylene

Table 15. Effect of mold inhibitors on the respiration of moist wheat. (125 g samples of "W" wheat tempered to about 80% moisture; trial conducted for six days.)

Sample number	Treatment for 100 g grain	Respiration mg CO <sub>2</sub> /100 g dry matter	Germination after trial	Moisture after trial	Fat after trial	
		Mean	after trial	after trial	after trial	
		daily rate: for 6 days	per cent			
I	None	134.	530.	44	21.2	60.3
II	0.5% Propylene oxide	15.0	53.0	0	20.5	41.5
III	0.5% 2 amino 3 methyl 1,3 propanediol	71.3	359.	72	21.0	41.5
IV	0.5% chlorotrifluoro-ethylmethyl ether	69.1	390.	73	20.9	60.6
V	0.5% ethionine	75.0	390.	64	21.2	59.3

Table 16. Effect of mold inhibitors on the respiration of moist wheat. (100 g samples of "D" wheat tempered to about 20° moisture; trial conducted for six days.)

sample number	Treatment	Respiration mg			Germination			Moisture			pH		
		CO <sub>2</sub> /100 g dry matter	maximum	total	after trial	per cent	after trial	after trial	after trial	after trial	after trial	after trial	after trial
		daily rate	attained	for trial									
I	None	66.0	341.		72		19.3				29.1		
II	0.1% Propylene oxide	61.1	196.		0		19.1				27.1		
III	0.3% Propylene oxide	56.4	73.5		0		19.0				23.7		
IV	0.5% Thiocarbamide	76.7	259.		33		19.3				27.9		
V	0.5% Thiocarbamide	55.7	205.		35		19.9				26.9		
VI	0.5% Piperonyl cyclonene	57.0	202.		35		19.0				22.0		

Table 17. Effect of oxidizing gases on the respiration of moist wheat. (100 g samples of "B" wheat tempered to about 20% moisture; trial conducted for 11 days.)

Sample number	Treatment for 100 g grain	Respiration mg		Germination after trial	Moisture		Fat
		CO <sub>2</sub> /100 g dry matter	Maximum : Total		after trial	after trial	
			daily rate: for : attained : trial :	Per cent			
I	None	73.1	653	27	19.8		40.5
II	HCl <sub>3</sub> , 0.087 g	67.9	562	23	19.7		35.6
III	HCl <sub>3</sub> , 0.166 g	60.3	503	23	19.8		36.7
IV	ClO <sub>2</sub> , 0.345 g	70.3	573	25	19.5		30.2
V	ClO <sub>2</sub> , 1.723 g	60.4	535	23	19.7		37.5

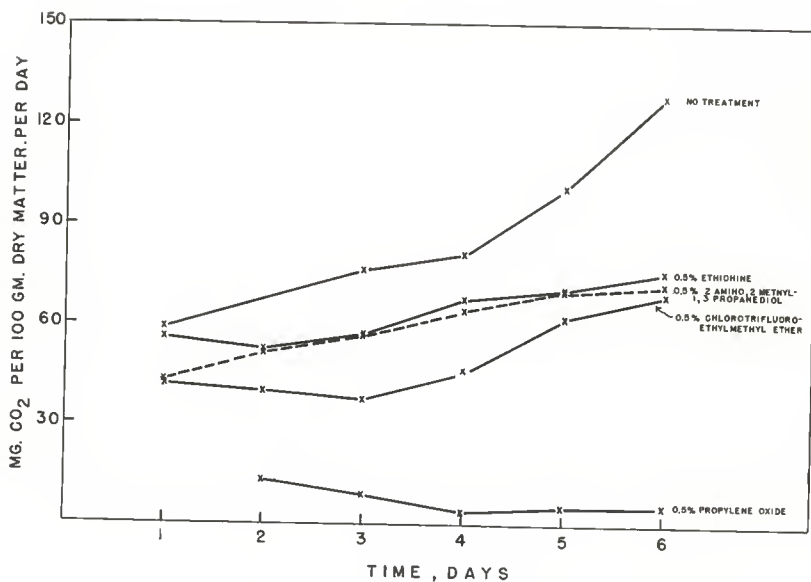


Fig. 8. Effect of mold inhibitors on the respiration of moist wheat.



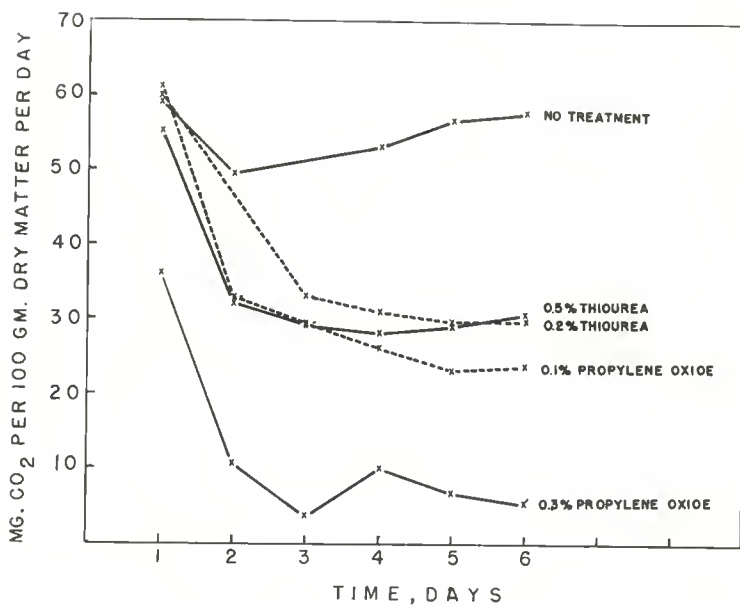


Fig. 9. Influence of mold inhibitors on the respiration of moist wheat.

oxide was by far the most effective mold inhibitor among the four studied in the first trial, if respiration is taken as a measure of mold activity. It did not seem to be quite as effective in reducing the development of free fat acidity as was othionine.

Reference to Table 16 and Fig. 9 indicates that confirmatory results were obtained in the second trial of this series in so far as propylene oxide is concerned. An increase of effectiveness with increase in concentration was observed with both propylene oxide and thiocarbamide. Propylene oxide was again observed to be far more effective than equivalent concentrations of any of the other chemicals employed. In this case, the superior effectiveness of propylene oxide was apparent both in the respiration values and the free fat acidity values.

Reference to Table 17 indicates that the common maturing agents Byox and Agene (chlorine dioxide and nitrogen trichloride, respectively) are also somewhat effective as mold inhibitors. However, the concentration of these chemicals was much greater than that usually employed in flour treatment. The effectiveness of the compounds did not increase greatly with an increase in concentrations in the range considered in this trial, and the inhibitory action is not nearly as drastic as that of propylene oxide.

#### Effect of Propylene Oxide on the Germminability of Wheat.

In view of the effectiveness of propylene oxide in preventing the molding of damp wheat, it seemed advisable to extend the

data for the toxicity of this substance to wheat. Some of the previous experiments had indicated an apparent increase in the susceptibility of the wheat embryo to toxic substances with increasing moisture content, and for this reason this work was conducted with wheat at various moisture contents.

Samples of "B" wheat were brought to moistures of 15 per cent and 20 per cent. Each of these samples was divided into two lots and one lot was treated with 0.2 per cent propylene oxide while the other was left untreated. Samples of the original wheat were treated with 2.0 per cent propylene oxide and 0.2 per cent propylene oxide. All of these were stored at room temperature. At intervals of two or three days samples were withdrawn from each lot and submitted for germination tests. Data secured in this experiment are presented in Table 19.

Reference to Table 19 indicates that low moisture content is not an efficient protection against the killing action of propylene oxide. Even at 11 per cent moisture almost 90 per cent of the viable kernels were killed within two days by a 0.2 per cent concentration of the chemical. This amount of propylene oxide is at the lower limits of the effective concentration for preserving grain from mold growth. This clearly indicates the inadvisability of applying propylene oxide to wheat intended for use as seed. Also indicated is the commonly observed phenomena of germination decrease with prolonged storage of untreated wheat at high moisture contents.

Storage Tests. In order to extend the investigation of the

Table 13. Effect of propylene oxide on the germinability of wheat. ("B" wheat, original germination 96 per cent.)

Treatment	Per cent germination				
	After 11 days of storage				
	3	4	6	9	11
11.1% moisture, 2% propylene oxide	2	0	0	2	0
11.1% moisture, 0.2% propylene oxide	10	8	12	5	7
11.1% moisture, no further treatment	96	-	-	-	97
15% moisture, no further treatment	97	87	94	92	95
15% moisture, 0.2% propylene oxide	0	0	0	0	0
20% moisture, no further treatment	96	92	83	81	63
20% moisture, 0.2% propylene oxide	0	0	0	0	0

action of mold inhibitors to include the effect of such chemicals on the milling and baking characteristics of wheat and to determine their effectiveness in inhibiting the changes in damp wheat when stored with a plentiful supply of oxygen, it was decided to apply several of the inhibitors which had shown most promise in preliminary trials to samples of moistened wheat large enough for milling on a Bühler experimental mill and to store them in containers which would permit intermittent aeration. The procedure followed is described in "Materials and Methods". Samples were examined and were milled and baked at four-week intervals over a period of 16 weeks.

Results obtained in tests on the samples withdrawn at the 4, 8, 12 and 16 week periods are shown respectively in Tables 19, 20, 21, 22, 23, 24, 25 and 26.

Reference to Tables 19, 20, 21, 22, 23, 24, 25 and 26 indicates that a constant decrease in moisture content occurred throughout the 16-week period in most samples. The final moistures averaged about three per cent less than the initial moisture values. The decrease in moisture percentage seemed to be greatest in the second and the fourth four-week periods and relatively slower at other times. This gradual drying of the samples is undoubtedly due to the effects of aeration, which swept out the moisture-laden atmosphere in the jars and replaced it with dryer gas. It might be expected that this tendency toward drying would slow down the degenerative changes associated with higher moisture contents, causing the greatest







Table 20 (concl.).

Heat treatment	Absorption	Dough	Formula	Volume	Specific	Texture	Appearance	Internal	Bread characteristics		
									Score	Color	Odor
Propylene oxide	60.3	Basic	Basic	4.71	4.71	Poor	Poor	85	84	Bl. gray	Musty
		Bromate	Bromate	4.76	4.76	Fair	Fair	80	82	Gray	Musty
		Rich	Rich	5.51	5.51	V. Good	V. Good	87	85	Yellow	Normal
Nitrogen atmosphere	60.4	Basic	Bromate	5.14	5.42	Fair	Good	87	82	Gray	Slightly musty
Oxygen atmosphere	60.4	Basic	Bromate	4.82	5.25	Fair	V. Good	85	83	Gray	Slightly musty
Dry control	60.4	Basic	Bromate	4.53	5.41	Poor	Fair	87	87	Creamy	Normal
		Rich	Rich	5.45	5.45	Fair	Fair	80	84	Yellow	Normal

Table 21. Effect of storage and mold inhibitors on properties of wheat, flour and bread.  
(Storage period of eight weeks at moisture value indicated. Inhibitors applied at rate of 5 ml (or g) per 5 kg damp wheat.) Wheat and flour characteristics.

Heat treatment	mois- ture %	acid- ity	% damaged (federal inspection)	Germl- ination %	heat appear- ance	exten- sion	mill- ing	absorp- tion	color- meter	flour odor	flour mois- ture
Ethylene chlorohydrin Thiourea	15.6 15.0	50.8 52.7	3.4 1.2	1 44	Good Dis- colored	70.0 70.0	53.3 61.0	65 57	65 57	Musty Musty	14.0 13.3
Piperonyl cyclonene	15.6	50.9	1.0	35	Very dis- colored	70.5	61.6	59	59	Musty	13.4
50% Propylene oxide in CCl <sub>4</sub>	15.2	49.0	2.6	1	Very dis- colored	70.2	61.4	66	66	peppery	13.6
Carbon tetrachloride	15.1	35.4	2.6	61	Dis- colored	70.9	61.0	63	63	Musty	13.5
Damp control	15.7	47.2	1.0	36	Very dis- colored	70.0	62.4	60	60	Musty	13.4
Propylene oxide	15.5	42.3	1.0	0	Good	69.0	61.4	66	66	Not mal	13.4
Nitrogen	15.3	54.1	1.8	34	Very dis- colored	70.1	59.8	64	64	Strong	13.3
Oxygen atmosphere	15.6	54.9	1.1	34	Dis- colored	70.0	59.8	58	58	Strong	13.6
Dry control		26.0	0	90	Very good	70.0	60.4	55	55	Musty Not mal	13.6



Table 22. Effect of storage and mold inhibitors on properties of wheat, flour and bread. (Storage period of eight weeks at moisture value indicated. Inhibitors applied at rate of 5 ml (or g) per 5 kg damp wheat.) Dough and bread characteristics.

Wheat treatment:	absorp- tion	:g/g:	:g/g:	:g/g:	:g/g:	:g/g:	:g/g:	:g/g:	:g/g:	Bread characteristics		
										Grain: appearance:	Grain: score:	Odor
Ethylene chlorohydrin	59.3	Basic	3.91	Poor	73	70	Creamy					Slightly musty
	57.9	Promote	5.49	Good	85	75	Creamy					Slightly musty
Thiourea	61.0	Basic	3.37	Poor	65	65	Gray					Slightly musty
	60.0	Promote	4.63	Fair	70	70	Gray					Slightly musty
Piperonyl cyclonene	61.6	Basic	4.57	Poor	70	70	Gray					Slightly musty
	60.6	Promote	3.86	Very good	80	74	Gray					Slightly musty
50% Propylene oxide in CCl <sub>4</sub>	61.4	Basic	4.94	Fair	73	65	Gray					Mustard
	60.4	Promote	5.70	Good	80	70	Gray					Slightly musty
Carbon tetrachloride	61.0	Basic	5.14	Good	80	75	Slightly gray					Normal
	60.0	Promote	5.73	Good	83	80	Slightly gray					Normal
Damp control	62.4	Basic	4.44	Poor	68	65	Gray					Slightly musty
	61.4	Promote	5.90	Very good	85	80	Slightly gray					Slightly musty
Propylene oxide	61.4	Basic	4.02	Fair	84	63	Slightly gray					Slightly musty
	60.4	Promote	5.94	Fair	83	73	Yellow					Slightly musty
Nitrogen atmosphere	59.8	Basic	4.02	Fair	70	69	Gray					Slightly acrid
	59.8	Promote	6.16	Very good	85	75	Slightly gray					Slightly acrid



Table 22 (concl.).

	: baking :	: read characteristics :			
heat treatment:	: absorb- : : tion :	: specific: : : iformal: : : volume :	: xternal: : : appear- : : ance :	: rain:fer- : : score: :	: color :



Table 23 (concl.).

	Final:	% damaged	Comal: Moist	Milling: value	Parino Graph	Flour
wheat treatment:	mois: acid:	Federal	moist: appear:	extrac: Absorp:	alori:	Flour: mois:
ture: ity:	inspection:	%	ance	ition	meter	odor: ture:
Oxygen atmosphere	15.3 66.7	2.0	25 dis-colored	70.0	60.3	66 duty 13.6
Dry control	10.4 22.2	0	91 Good	70.0	61.5	53 Nor- 13.5 mal

Table 24. Effect of storage and mold inhibitors on properties of wheat flour and bread.  
(Storage period of 12 weeks; inhibitors applied at rate of 5 ml (or g) per 3 g damp wheat; initial moisture 16.7%.) Dough and bread characteristics.

Inhibitor	Damp- ing : %	Heat treatment: absorp- tion : g	Dough : formaldehyde : %	Specific volume : g/cc	Dough appearance : grade	Bread formaldehyde : %	Bread appearance : grade	Bread color : description	Bread odor : description
Ethylene chlorohydrin	59.3 60.3	Basic Bromate	Basic Bromate	6.24 5.33	Fair Poor	90 90	85 82	White Cr. white	Normal Normal
Thiocarbamide	62.3 62.3	Basic Bromate	Basic Bromate	5.43 5.36	Poor Poor	80 85	80 85	Sl. grayish Grayish	Acrid Normal
Piperonyl cyclonene	62.3 63.3	Basic Bromate	Basic Bromate	6.55 6.53	Good Fair	85 80	90 87	Cr. white Cr. white	V. musty Musty
50% propylene oxide in carbon tetrachloride	59.3 60.3	Basic Bromate	Basic Bromate	6.23 6.74	Fair Good	80 85	87 80	Grayish Grayish	Normal Musty
Carbon tetra- chloride	59.3 60.3	Basic Bromate	Basic Bromate	6.73 6.64	Good Fair	85 90	85 90	Cr. white Cr. white	Musty Very sl. musty
Damp control	63.3 61.3	Basic Bromate	Basic Bromate	6.27 6.09	Fair Good	85 85	85 80	Cr. white Cr. white	Musty Musty
Propylene oxide	63.3 63.3	Basic Bromate	Basic Bromate	6.04 5.95	Poor Poor	80 85	80 82	Cr. white Cr. white	Sl. musty Sl. musty
Nitrogen atmosphere	63.3 61.3	Basic Bromate	Basic Bromate	6.04 6.32	Fair Fair	85 85	87 85	Cr. white Cr. white	V. musty Sl. musty







Table 25 (concl.).

	Mineral:	Investment:	Acid:	demanded:	Condi-:	Cont:	filling:	Permeability:	Flour:
Heat treatment:	mois-:	acid-:	federal:	inspection:	nation:	appear-:	extrac-:	absorp-:	Value-:
	ity:	ity:	ity:	ity:	ity:	ance:	tion:	tion:	Water:
	ity:	ity:	ity:	ity:	ity:	ity:	ity:	ity:	ity:
Nitrogen atmosphere	13.1	62.3	17.0	20	Very dis- colored	70.0	50.5	67	Very musty
Oxygen atmosphere	11.9	60.1	3.0	19	Very dis- colored	71.0	50.6	60	Very musty
Dry control	11.9	35.2	0	23	Good	72.0	62.	56	Slut-13.3 ly musty

Table 26. Effect of storage and mold inhibitors on properties of wheat, flour and bread.  
(Storage period of 16 weeks; inhibitors applied at the rate of 5 ml (or g)  
per 5 g damp wheat; initial moisture 16.7%.) Dough and bread characteristics.

Wheat treatment:	absorp- tion %	Dough formula:	volume	specific gravity	external appearance	Grain score:	Inhibitor score:	Color	Odor
Ethylene chlorohydrin	50.8 50.8	Basic Bromate	5.64 5.37	Good Fair	35 37	35 32		Creamy white Creamy white	Normal Musty
Thiocarbamide	50.8 59.8	Basic Bromate	4.49 4.71	Fair Poor	30 30	30 35		Slightly gray Grayish	Musty Musty
Piperonyl cyclonono	52.8 53.3	Basic Bromate	5.35 6.08	Good Poor	35 30	30 37		Grayish Grayish	Very musty Musty
50% propylene oxide in carbon tetrachloride	50.8 50.8	Basic Bromate	6.01 6.20	Poor Poor	30 32	35 30		Grayish Grayish	Musty Musty
Carbon tetra- chloride	50.3 50.8	Basic Bromate	5.45 6.73	Fair Fair	37 37	35 30		Grayish Slightly gray	Musty Musty
Damp control	51.8 51.8	Basic Bromate	4.79 6.16	Fair Fair	35 35	30 32		Grayish Slightly gray	Musty Musty
Propylene oxide	53.8 53.0	Basic Bromate	4.93 5.67	Good Poor	35 30	37 35		Grayish Grayish	Musty Musty
Nitrogen atmosphere	51.8 51.8	Basic Bromate	5.34 5.19	Poor poor	32 35	30 35		Grayish Slightly gray	Musty Musty

Table 26 (concl.).

Heat treatment	: taking : : absorp- : : tion :	: rough : : formula : : g :	: volume : : : : :	: specific : : : : :	: normal : : : : :	Local characteristics			
						: appear- : : : : :	: score : : : : :	: Color : : : : :	: Odor : : : : :
Oxygen atmosphere	60.3 60.3	Basic Bromate	5.42 6.02	Good Good	87 80	90 90	Grayish Grayish		Musty Musty
Dry control	60.3 61.3	Basic Bromate	5.37 7.02	Fair Good	85 80	80 87	Creamy white Creamy white		Normal Normal

deleterious effects to be observed in the first four-week period with a gradual lessening of the rate of deterioration throughout the succeeding periods. Opposing this effect would be the increasing mold spore content, which would probably be greater at the start of each successive four-week period. Facilities for germination and growth of the mold spores, however, would be lessened by the factors previously mentioned. Total decrease of moisture content in all of the samples was much the same with the exception of the untreated sample stored under oxygen atmosphere and the final moisture content value given for this sample can be regarded as doubtful.

Fat acidity values increased regularly throughout the first three storage periods, but these values appeared to be lowered slightly during the final four weeks. The reason for the latter fact is not known. Although a relatively stable fat acidity value could be expected as the wheat became dryer, a regression in the values was not expected. Perhaps the molds began to utilize the fatty acids in their metabolism as the mold population increased and competition for nutrients became greater. Ethylene chlorohydrin was the chemical found to be most effective in holding down the development of free fat acidity. The final values for the sample of wheat treated with this substance were about a third lower than the value for the oxygenated damp control. Thiocarbamide and propylene oxide were also very effective in this respect.

The external appearance of the samples became more and more



undesirable as the length of the storage period increased. Most of the wheat became discolored and darkened after four weeks storage and at the 16 week examination all of the samples were discolored, except of course, the dry control. At this time the wheat smelled musty and, in many cases, gave off a dense cloud of mold spores when poured from the jar. Ethylene chlorohydrin was relatively effective in preventing development of discoloration and moldy odors, and propylene oxide delayed the development somewhat. The number of damaged kernels in the samples increased steadily and the commercial grade decreased in proportion. The control was graded "No. 1 Hard Dark Winter" at the start of the storage period, but after 16 weeks of storage in a damp condition the wheat had deteriorated to Sample Grade. Propylene oxide, thiocarbamide and 80 per cent propylene oxide in carbon tetrachloride were found to be the most effective treatments to reduce the rate at which the percentage of damaged kernels increased.

Propylene oxide, 1, 3 dichloropropene-1, and 50 per cent propylene oxide in carbon tetrachloride reduced the wheat samples so treated to zero germination at the end of the first four week storage period. The germination of the other samples decreased less rapidly but at the end of 16 weeks all of them had germinations less than a third of that of the original sample. The decrease was constant and regular. Carbon tetrachloride was the most effective chemical in preserving germination in the damp grain.

There did not seem to be any significant difference in milling extraction or farinograph absorption between the various samples at the beginning of the tests or after the final storage period. Nor did the individual samples change in regard to these two characteristics. The valorimeter values however, increased an average of 10 per cent during the 16 weeks. This increase was regular and constant throughout the first three storage periods but was slight or insignificant during the final four week period. Also apparent was a decided change in curve characteristics, from a slowly developing farinogram with a curved top and moderately rapid breakdown for the flour from the dry control to a rapidly developing, straight-topped farinogram suggestive of a strong flour with great mixing tolerance which was obtained with the flours from most samples stored damp for 16 weeks and aerated with oxygen. The greatest valorimeter values were obtained for the flours from samples treated with carbon tetrachloride and propylene oxide. The dry control had an absorption value which remained essentially unchanged and the value was about two per cent higher than the damp stored samples after 16 weeks of storage.

With the exception of the sample treated with carbon tetrachloride, all wheats gave flour that was musty in odor or had an odor similar to that of the chemical with which it was treated after only four weeks of storage. After 16 weeks, all of the flours were decidedly musty in odor. Wheat treated with 1,3 dichloropropene-1 gave flour that was foul and nauseous in

odor.

After the first four weeks baking tests it became obvious that the formula which included dried milk ("rich") would not provide information not afforded by the loaves baked from the "Bromate" formula, and consequently the "Rich" formula was not used in subsequent baking tests. In general, the specific volume of the loaves increased as the test periods proceeded. Occasional aberrant results must be ascribed to experimental error. The bromate response seemed to decrease steadily, except in the case of the dry control which seemed to increase its bromate response as the experiment proceeded. Thiocarbamide reduced loaf volume considerably.

Loaf characteristics such as texture, grain, and external appearance showed no definite trends, except that, in most cases the damp wheat gave flour that was considerably inferior in baking quality to that from the dry control. Propylene oxide, carbon tetrachloride, and ethylene chlorohydrin seemed to be the most effective in preserving desirable baking qualities.

In nearly all cases, the musty or chemical odors noticeable in the flours were transmitted to the loaves baked from them. In the case of wheat treated with 1,5 dichloropropene-1, the odor transmitted to the loaves baked after four weeks storage was so thoroughly repulsive as to eliminate this chemical from further consideration as a preservative.

The mustiest wheat gave flour which caused a grayish color in the crumb of loaves baked from it. At the end of 16 weeks

all the loaves except the ethylene dichlorohydrin treated sample had this appearance.

In resume, it can be said that propylene oxide and carbon tetrachloride, alone or in combination, seemed to be more effective than any other chemical tested in preserving the desirable qualities of wheat for the milling trade. Even these chemicals, however, when applied in the manner used in this study, did not entirely prevent the deterioration of high moisture content wheat upon prolonged storage.

#### Effect of Mold Inhibitors on the Respiration and Associated Characteristics of Molded Wheat

In order to obtain further information on the effect of various mold-inhibiting chemicals on the respiration of wheat at high moisture contents, some experiments were undertaken with damp wheat that had been stored at room temperature in a closed container for several days before being employed in the respiration trial. As a consequence of this treatment, the samples of wheat were all visibly infected with a heavy growth of mold before being placed in the respirometer. Various moisture contents were used and the samples were allowed to mold for varying periods of time before use in order to get a more complete idea of the influence of the different factors which affected the final result. The chemicals used were the commonly used flour bleaching gases nitrogen trichloride and chlorine dioxide as well as carbon tetrachloride, 1,2,3 trichloropropane



and trichloroethylene.

The results of this experiment are presented in Tables 27 and 28 and Fig. 10.

Reference to Table 27 and Fig. 10 indicates that both carbon tetrachloride and trichloroethylene were quite effective in reducing the rate of respiration of the grain, and that trichloroethylene was slightly more efficacious than carbon tetrachloride in this respect. 1,2,3, trichloropropane apparently had no effect on the respiratory rate. In contrast to the results obtained in the experiments on freshly dampened grain, an initial application of liquid chemical reduced the respiration of the presoaked wheat considerably more than did continuous application of the vapor of the chemical. In general, the lowest fat acidity values were associated with a low rate of respiratory activity and high fat acidity values were associated with a high respiratory activity, a result which had been observed in previous experiments with freshly dampened grain.

Reference to Table 28 indicates that a considerably higher respiratory rate was observed for grain which was slightly damper and which had been stored longer in the moistened state than the wheat that had been utilized in the experiment described above. Both of the gases used, nitrogen trichloride and chlorine dioxide, lowered the total respiration values significantly at the lowest concentrations employed, and the rate of inhibition was greater at higher concentrations, though not proportionately so. In one instance, the maximum rate attained was somewhat



Table 27. Effect of certain mold inhibitors on the respiration of molded wheat.  
(100 g samples of "B" wheat which had been molded for 14 days at  
about 20% moisture; trial conducted for 10 days.)

Sample number	Treatment	Respiration mg		Moisture		Fat	
		Co <sub>2</sub> /100 g dry matter	Co <sub>2</sub> /100 g dry matter	after trial	after trial	after trial	after trial
		maxima	total	per cent	per cent	per cent	per cent
		daily rate	for attained	trial	trial	test	test
I	None	60.0	542	59	19.3	42.8	42.8
II	1% CCl <sub>4</sub>	47.8	316	32	19.0	40.3	40.3
III	Continuous tri- chloroethylene vapor	45.3	415	40	19.2	32.9	32.9
IV	1% trichloroethylene	33.0	252	13	19.2	34.1	34.1
V	Continuous 1,2,3 trichloropropane	64.0	530	12	19.1	43.5	43.5
VI	Continuous CCl <sub>4</sub> vapor	45.3	367	19	19.0	35.0	35.0

Table 29. Effect of nitrogen trichloride and chlorine dioxide on the respiration of molded wheat. ("B" wheat allowed to mold for 17 days at about 20% moisture; trial continued nine days.)

Sample number	Treatment per 100 g dried grain	Respiration mg CO <sub>2</sub> /100 g dry matter		Moisture	
		Maximum daily rate attained	Total for trial	after trial per cent	Free fat acidity
I	None	123	1056	19.7	77.7
II	37.3 Mg NCl <sub>3</sub>	79.2	651	19.4	67.3
III	136 Mg NCl <sub>3</sub>	69.3	547	19.4	62.9
IV	0.345 g ClO <sub>2</sub>	132	647	19.6	69.8
V	1.73 g ClO <sub>2</sub>	80.4	645	19.5	66.4
VI	1 g CCl <sub>4</sub>	68.2	469	19.4	69.1

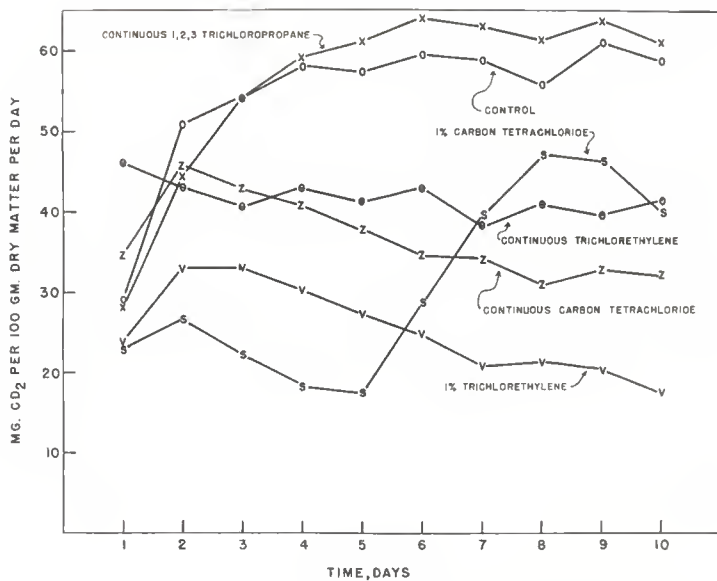


Fig. 10. Effect of certain mold inhibitors on the respiration of molded wheat.

higher for the treated sample than for the control, although the total carbon dioxide respired was considerably less than for the control sample. The reason for this apparent discrepancy is not known. Fat acidity values increased with increases in the total carbon dioxide respired.

In both of the experiments described above there was noted a great decrease in germination due to the mold growth and a further decrease occurred as a result of the application of every chemical studied.

#### DISCUSSION

The data obtained in these experiments reveal that few, if any, changes of significance occurred when wheat was treated with ethylene. Negative results were obtained when the wheat so treated was dry, moist, mature or immature, and when widely different dosages of ethylene were employed. Factors observed included respiration, germination, development of free fat acidity, and baking properties. Although it is well known that ethylene is remarkably powerful in causing alterations in the growth and maturation of certain seedlings, flowers and fleshy fruits, its effect on dormant grains is obscure. The data herein presented would tend to indicate that the gas is physiologically almost inactive insofar as the wheat kernel is concerned.

Carbon tetrachloride was shown to be an effective fungistatic agent when applied to damp wheat in sufficient quantities.

Tables 9 and 10 and Fig. 5 illustrate well the inhibitory action which the chemical has on changes associated with mold growth in moist grain. This phenomenon is not surprising when it is considered that carbon tetrachloride, like many other chlorinated hydrocarbons, acts as a cell poison when present in great enough concentrations. The chemical is effective only as long as it remains present in the grain in appreciable quantities, and it has no residual effect. Thus it appears that the mold spores are quite resistant to actual killing by the chemical, and are only anesthetized by it. Carbon tetrachloride was found to lose some of its effectiveness when the moisture content of the grain was increased to high levels, a further indication that the method of action of the chemical is as an anesthetizing agent which may be overcome if conditions of growth are sufficiently good.

A large number of chemicals were tested in a preliminary manner for their relative effectiveness in inhibiting mold growth in damp grain as measured by free fat acidity, visible mold decrease in germination, etc. As indicated in Table 13, several of these gave favorable results and some were selected for further study. Most of the chemicals tested were disqualified for various reasons, and several compounds known to be effective fungistats in certain specialized fields were found to be of little or no value when applied to damp wheat. This, of course, emphasizes the special problems encountered in the study of grain preservatives. The fungi normally inhabiting grain are



no doubt specialized saprophytes possessing highly individual growth and adaptation characteristics not possessed by other species infesting other commercially important products. Ineffective compounds were found to vary widely in chemical structure, but, as a class, the small molecular weight chlorinated hydrocarbons, especially those with a double bond, seemed to have an unusual degree of toxicity for the molds concerned. They also usually had a high degree of toxicity for the wheat embryo. Both propylene oxide and ethylene oxide were found to be effective in inhibiting the growth of fungi and the former was especially effective. This is not surprising, since other investigators have shown the epoxides to be effective fungistats when applied to certain other food products often infested with molds, such as figs and dates.

Some of the more favorable chemicals employed in the previous section were applied to moist wheat and the respiration of the wheat was measured, since it was assumed that the respiration rate of the grain was a more sensitive method of differentiating the fungi-inhibiting capabilities of the compound. This supposition was supported to a considerable extent by the data secured. Preliminary findings as to the relative effectiveness of the compounds were generally confirmed, but the respiration of the wheat was shown to be a much more reliable and sensitive indicator of the chemicals' effectiveness than was fat acidity or associated factors. Propylene oxide was, by far, the most efficient chemical studied. This is suggestive of

possibilities for commercial use, since propylene oxide is cheap, easy to apply, and hydrolyzes to leave an innocuous residue.

One disadvantage of propylene oxide is its great toxicity to the wheat embryo which was shown by the data in Tables 13, 14 and 15. To further elucidate this phenomenon, another experiment was performed and resulted in the data given in Table 17. This data confirms that obtained previously and indicates that the poisonous effect is more or less independent of the moisture content and occurs immediately upon application. This is indeed a serious barrier to use of the chemical on seed wheat, but would hardly affect its value as a preservative for grain destined for milling.

Some of those chemicals which showed possibilities of being commercially valuable as preservatives for damp grain were applied to comparatively large samples of wheat which were ventilated at frequent intervals with oxygen to provide supposedly optimum conditions for mold growth. Three sets of untreated controls were used; one of which was sealed, one of which was periodically aerated with oxygen and one of which was periodically flushed with nitrogen.

In general, all of the samples showed an increase in fat acidity and damaged ("sick") kernels, and a decrease in germination. The odor and appearance of the wheat became quite undesirable and it decreased from a grade of no. 1 hard dark winter to Sample Grade after 16 weeks of storage. There was a slow deterioration of baking quality of the flour milled from the

wheat, but, in general, the absorption and calorimeter values did not change much. The leaves baked at the end of the test period were gray in color and all had a very unpleasant musty odor. They were not considered edible.

Some of the chemicals employed imparted a very objectionable odor to the wheat, flour and bread. Particularly marked in this respect was 1,3 dichloropropene 1. Wheat treated with this chemical was discarded after two months storage, primarily because of the odor, which was considered sufficient to eliminate it from consideration for commercial uses.

An unexpected result was the change in farinograph curve characteristics. At the beginning of the experiment the flour milled from the control wheat gave farinograph curves of normal appearance for a moderately strong flour. However, after 16 weeks of storage most of the flours gave farinograph curves which showed a rapid development of the dough and a very slow break down without any great alteration in absorption. The mixing times for the bread doughs were also increased considerably. This mixing tolerance is usually indicative of a strong flour and would tend to show that the baking quality of the flour increased during the storage period. This, however, was not borne out by the performance of the flour when baked into loaves. The factors behind these observations can only be conjectured. Perhaps the protein structure of the flour was improved during storage by an alteration in the oxidation-reduction potential, which may have resulted either from enzymes

liberated into the wheat endosperm by the growing fungi, or from intrinsic changes in the wheat berry due to its own metabolic processes, or, more likely, from the high oxygen content atmosphere surrounding the wheat. Facts in support of the latter supposition are: the good baking qualities of the damp oxygenated control as compared with the damp nitrogen atmosphere control and the damp non-aerated control, and the low bromate response of the damp oxygenated control. The general decrease in baking quality from that of the dry control could be considered a result of the amylolytic and proteolytic changes caused by the mold and wheat enzymes. These could adversely affect the bread dough during the long fermentation period without appreciably affecting the farinograph dough in which fermentation is not a factor.

Of the chemicals studied, propylene oxide and ethylene chlorohydrin were most effective in hindering the visible deterioration of the wheat. Insofar as preservation of the baking qualities of the flour is concerned, results were largely inconclusive, but it appears that carbon tetrachloride and propylene oxide, mixed or separate, were most efficacious. No synergistic effect was observed when these chemicals were mixed.

A further series of experiments was undertaken to determine the effect of some of the chemicals previously studied on wheat which had become thoroughly moldy before treatment. As far as the respiratory rates were concerned, chemicals found to be depressants for freshly moistened grain acted in a similar manner



on moldy grain. As shown in fig. 10, the respiration tended to increase when aeration was begun. This is no doubt due to the removal of the accumulated waste gases which must have had an inhibitory effect on the vegetating mold. Observations in this series of experiments were largely analogous to those made with freshly moistened grain.

#### SUMMARY

Influence of ethylene on the respiration of wheat at various stages of maturity was studied with the objective of determining whether this gas might induce maturity in such grain. In addition, a large number of organic compounds were tested in a preliminary manner as preservatives for damp wheat. The influence of some of these compounds on the respiratory characteristics and quality of the wheat for milling and baking purposes was also investigated.

Data secured in experiments with ethylene rather definitely eliminate this chemical from further consideration as a possible wheat maturing agent. Those differences observed between wheat and flour treated with ethylene and the untreated controls which might be attributed to the action of the gas were minor and not of a type which would increase the commercial value of these cereal products.

Carbon tetrachloride was found to decrease the respiratory rate of moist wheat and to inhibit forms of deterioration that



are typical of mold growth damage without introducing other deleterious changes itself. No residual protective effect is obtained, however, with carbon tetrachloride. This compound can be considered as one of the most promising of those investigated as chemical preservatives.

Inhibition of development of free fat acidity in moist, treated wheat was found to be a good indication of the effectiveness of any compound as a mold inhibitor. By this method, in conjunction with tests for germination and physical appearance, 104 organic compounds were evaluated. Several of these compounds were observed to be sufficiently promising to merit more thorough testing. Among these were propylene oxide, thiocarbamide, piperonyl cyclonene and several of the lower molecular weight chlorinated hydrocarbons, especially those containing a double bond.

Propylene oxide was shown to be very toxic to grain even at fairly low concentrations. This effect was slightly more rapid on moist grain than on dry grain, and increased with a higher concentration.

Propylene oxide and carbon tetrachloride were shown to be among the most effective chemicals in preventing the deterioration in milling and baking quality of large wheat samples during extended period of storage with the exception that use of the former compound was accompanied by a fairly high percentage of damaged "sick" kernels. Baking quality of the flour from wheat stored for considerable lengths of time in a moist condition

deteriorated somewhat regardless of the mold inhibitory treatment employed. The most serious defects were the objectionable odor and gray color of bread from damaged wheat. Aroma response was reduced in all samples of the wheat stored at high moistures, but it was not appreciably reduced over a four-month period in wheat stored dry. The samples of wheat stored at the original low moisture content gave flour that was somewhat improved in baking quality after four months of storage. Storing moist wheat in a nitrogen atmosphere or in an air-tight container did not seem to reduce appreciably the rate of deterioration. Some of the chemicals employed, notably ethylene chlorohydrin and thiocarbamide, had a deleterious effect on the baking qualities of flour milled from wheat so treated.

In general, those chemicals such as carbon tetrachloride and trichloroethylene which were found to be effective inhibitors of mold growth on freshly-wetted grain were also found to be effective in preventing continued deterioration when applied to previously molded wheat. Nitrogen trichloride and chlorine dioxide were also effective in this respect, but not especially so. Changes which had occurred in the wheat previous to the application of the chemical were not reversed by the treatment.

## ACKNOWLEDGMENTS

Acknowledgment is extended to Dr. Max Milner, major instructor, for invaluable aid in directing the investigation and checking the manuscript and to other members of the Department of Milling Industry without whose cooperation this investigation could not have been completed. Thanks are also due to Anna Becker, seed analyst, for carrying out the germination tests.

The author is indebted to General Mills, Inc., Minneapolis, Minnesota, for the financial assistance which made this study possible.

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