MICRO-ORGANISMS ASSOCIATED WITH THE SELF-HEATING OF DAMP GRAIN

by

A. R. Lejeune, B.Sc.
The University of Manitoba

A Major Thesis submitted to the Committee of Graduate Studies and Research of The University of Manitoba in candidacy for the Degree of Master of Science

1950

ACKNOWLEDGEMENTS

The writer expresses his appreciation to Dr. Norman James, Chairman, Department of Bacteriology and Animal Pathology, for advice, assistance and honest criticism during the investigation.

The writer is also grateful to Kenneth I. Roulston, Assistant Professor of Physics, for aid in construction and maintainance of electronic equipment used in the study.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

INTRODUCTION

Self-heating has long been recognized as a major problem associated with the storage of damp grain. Moisture, temperature, aeration, volume of storage piles and condition of grain are contributing factors. However, it is accepted that the fundamental cause of self-heating is biological and not physical, although controversy continues as to the type of biological activity responsible. It is now generally believed that neither respiration of dormant grain nor germination produces sufficient heat to raise the temperature to that observed in self-heating grain; and that, although insects may be involved in a few cases, the main cause of self-heating is micro-organic activity.

This study was undertaken to obtain further evidence on the subject by determining relationship, if any, between the microflora on grain and self-heating in an adiabatic chamber.

HISTORICAL

Since self-heating of grain is accompanied by high respiration rates, much work has been done on the respiration of grain. Bailey and Gurjar (3) found that wheat

which was artificially conditioned to 12% moisture or more had high respiration rates and that respiration increased with time. Increases in temperature, up to 50°C., were accompanied by increases in respiration. These workers also found that increases in the CO_2 content of air surrounding the grain were associated with decreases in respiration and that poor quality grain (cracked, weather, frozen kernels, etc.) had high respiration rates. Later, Bailey (2) in experiments on the effect of moisture, aeration and quality on the respiration of barley, oats, rye and flax obtained results similar to the above. Swanson (19) observed that temperature, moisture and aeration were associated with rancidity and visible moldiness of wheat. Robertson, Lute and Gardner (17) showed that a relationship existed between relative humidity and respiration of wheat, oats and barley. Bakke and Noecker (1) observed that respiration of oats varied with moisture content. James (7), using cracked corn and James, Rettger and Thom (8), using hay and cornmeal, found that oxygen was necessary for self-heating. Both Leach (12) and Oxley (13) have shown that removal of the embryo, the most active part of the wheat kernel, did not significantly reduce the respiration rate. Gilman and Barron (6) found that germination of wheat, which had been previously exposed to a sterilization (disinfection) treatment, produced only a slight rise in temperature. Thus it is evident that metabolism of the viable seed cannot ac-

count for all the increase in temperature encountered in self-heating.

Oxley (13,14) found that insects were associated with the heating of wheats with moisture contents between 11% and 14%. He reported that this "dry grain" heating did not produce temperatures above 42°C. and caused only slight deterioration of the grain, except at the surface. However, he believed that "dry grain" heating may raise the moisture content sufficiently to encourage typical damp grain heating.

James (7) obtained temperatures up to 62°C. with moistened cornmeal in insulated Dewar flasks. Larmour, Clayton, and Wrenshall (11) found that the addition of 100 gm. heated wheat to a fifteen pound sample of damp wheat decreased the time required for the latter to reach a maximum temperature; and that wheat kept in carbon tetrachloride vapor (a fungicide) showed no increase in temperature even at high moisture contents. These workers believed that these findings constituted evidence that fungi were responsible for self-heating. Robertson, Lute and Gardner (17) observed fungal growth on wheat stored under conditions of high relative humidity.

Pierce (15), testing the heat production of germinating peas, found that samples reaching temperatures of 33.7°C. to 53°C. were rotted at the end of the trial; while samples not rising above 30°C. remained sound. In similar experiments, Darsie, Elliot and Pierce (5) found that a sample which rose to an abnormally high temperature was infected

with a species of Mucor.

Leach (12) stated that when the relative humidity of the air surrounding grain was maintained at about 92%, (equivalent to grain moisture of about 25%) the rapid rise in CO_2 production was the "result of respiratory activity of the fungi present." Oxley (13) found considerable fungal mycelium on the inner surface of the epidermis of wheat. He reported the belief that the presence of mycelium was responsible for the high respiration rate at the lower relative humidities (70% or less). Gilman and Barron (6) obtained high temperatures with sterilized wheat inoculated with Aspergillus niger, A. flavus or A. fumigatus. The maximum temperatures were 10° to 20° lower in wheat at 18% moisture than in samples of the same wheat conditioned to 20% moisture. Bakke and Noecker (1) found samples of self-heating oats infected with Aspergillus niger. One sample which rose to 35°C. had 100% of the kernels infected while a sample reaching 27° had only 5% infected. James, Rettger, and Thom (8) made bacterial and fungal counts on heating corn. Bacterial counts increased as the temperature of the corn increased, up to 50°C., and then decreased up to the maximum temperatures (usually between 55° and 62°C.) Fungal counts remained steady up to 50°C. and then decreased about as did the bacterial counts. One sample had a fungal count of 500,000 per gm. The fungus was found to be Aspergillus fumigatus. This sample reached a maximum temperature of

62.3°C. It remained between this temperature and 60°C. for fourteen days and dropped to 52°C. on the fifteenth day. This species, and other species of micro-organisms isolated from self-heated corn, were inoculated separately into sterilized corn. Four strains of Bacillus subtilis, one of B. vulgatus, and one of Proteus vulgaris, as well as Aspergillus fumigatus, produced temperatures of 50°C. or higher in inoculated samples. Using adiabatic equipment, Sallans, Sinclair and Larmour (18) obtained temperatures up to 52.1°C. with flax and sunflower seed and Ramstad and Geddes (16) obtained temperatures up to 88.5°C. with soybeans. The last mentioned workers believed the microflora to be associated with self-heating.

PRELIMINARY EXPERIMENTS

In order to establish the relationship between micro-organisms and self-heating, it appeared desirable to have check samples of viable grain free from micro-organisms. Several methods were tried in an effort to obtain such samples.

Following the method suggested by Oxley (13) a 300-gm. sample of wheat was placed in a sterile quart sealer together with 300 gm. of sterile carborundum crystals slightly smaller than wheat kernels. The sealer was placed on a revolving mixer designed to accommodate the quart sealer and geared to revolve 40 to 50 times per minute.

After 4 hours, the sealer was removed and the glass lid replaced by a sterile brass lid perforated with holes slightly smaller than wheat kernels but larger than carborundum crystals. The carborundum was removed by shaking the inverted sealer. The grain was then washed 12 times. Each washing consisted of manual shaking to and fro 50 times with about 300 ml. sterile water. The wheat kernels and wash water, even after 12 washings, still showed the presence of carborundum dust and harboured about the same bacterial and fungal populations as the check. The results are presented in Table 1.

Table 1. Effect of the removal of bran layer with carborundum on the numbers of organisms on wheat.

(Average count per gm. from 4 plates)

	Bacteria	Yeasts	Fungi
Normal Sample	400,000	47,000	1,200
Treated Sample	330,000	1,800	1,000
Reduction in %	17	96	18

An attempt was made, next, to remove the waxy substance on the surface of the grain and with it probably large numbers of micro-organisms. A fifty gm. sample of wheat was washed with 100 ml. xylol on the revolving mixer

for 30 minutes, the sample was then rinsed 12 times with 100 ml. amounts of sterile water and plated. The sample showed no bacteria on any plate at a 1:200 dilution, while fungal counts ranged from 0 to 5 on the four plates at the 1:2 dilution. However, only 73 out of 100 seeds tested for germination were viable.

According to James, Wilson and Stark (9) the number of micro-organisms on wheat can be appreciably reduced by repeated washings with sterile water.

A pilot 50 gm. sample was given 12 five-minute washings in 100 ml. amounts of sterile water. The number of bacteria on the wheat was reduced by 89% and the number of fungi by 95%. One hundred per cent of 100 seeds tested, germinated. A trial was then made with larger samples since at least 700 gm. were needed for an experiment in the adiabatic chamber. Two 350-gm. samples were washed in the same way as the 50-gm. sample with amounts of sterile water equal in volume to the sample. The two samples were then thoroughly mixed together. Ninety-six per cent of 100 seeds tested, germinated. Counts of bacteria and fungi were comparable to those of the 50-gm. sample as shown in Table 2.

Table 2. Effect of washing in sterile water on the numbers of organisms on wheat.

(Average count per gm. from 4 plates)

	Bacteria	Fungi
Normal Sample	400,000	1,200
Washed Sample, (700 gm.)	79,000	60
Reduction in %	80	95

From the results of these experiments it appeared evident that repeated washing in sterile water was a satisfactory method for preparing check samples for this study.

Washed samples were spread over sheets of heavy, perforated cardboard, which had previously been wiped with a cloth soaked with a disinfectant, and dried in a 50°C. or a 37.5°C. incubator. It was found that a moisture content of between 20% and 25% was obtained in 30 to 40 minutes at 50°C. or in 60 to 70 minutes at 37.5°C.

The results of typical trials are shown in Table 3.

Table 3. Effect of temperature and time of drying on the moisture content of freshly-washed wheat.

Temperature °C.	Time Minutes	Moisture %
50	30	22.07
50	40	24.20*
50	60	7.50
37.5	60	22.20
37.5	70	23.26*

* The apparent discrepancy in these results undoubtedly was due to the use of different samples in the different tests and to the difference in moisture on the samples before drying.

MATERIALS AND METHODS

Ten pound samples of Thatcher and Marquis wheat from Melfort, Saskatchewan, 1947, and of the same varieties from Lethbridge, Alberta, 1949, were obtained from the Dominion Laboratory of Cereal Breeding, located on The University of Manitoba campus. These were called Thatcher 47, Marquis 47, Thatcher 49 and Marquis 49, respectively.

Moisture Determinations

Moisture determinations were made by air drying two-gm. samples in an oven at 135°C. for 2 hours. The figures used were averages of four replicate determinations.

Germination Tests

One hundred replicate kernels from a well-mixed sample were placed in Petri dishes, 10 kernels per dish, between moistened blotting paper. After 7 days the percentage of kernels germinating was determined.

Isolations from Wheat

Estimates of the number of bacteria and fungi on wheat were obtained by the following method. Ten gm. replicates from a well-mixed sample were transferred aseptically to 6 oz. screw-cap bottles, each containing 100 ml. sterile water and 10 gm. sterile, fine gravel. These 1:10 dilutions were then shaken on a mechanical shaker for 30 minutes. Duplicate plates were prepared from appropriate dilutions made from the 1:10 dilutions. Nutrient agar was used for bacterial counts and Czapek's agar and Malt agar for fungal counts. Incubation was at 25°C. for 6 days and at 37.5°C., and 50°C. for 2 days.

Moisture Conditioning

A 700-gm sample, of which the moisture content had previously been determined, was placed in a sterile two-quart sealer. The amount of water necessary to bring the sample to the desired moisture was calculated and added directly to the sample. The lid was screwed on tightly and the sample mixed on a mechanical mixer for 30 minutes. The sample was then placed in a refrigerator at about 9°C. Once every day the sample was remixed for a 10-minute period. Moisture determinations showed that the moisture was evenly distributed and constant after three days.

The Adiabatic Chamber and its' Operation

Although some workers have obtained high temperatures with self-heated grain in Dewar flasks held at constant temperatures, it was believed that more accurate information could be obtained by using adiabatic equipment. An adiabatic chamber, similar to that used by Ramstad and Geddes (16) was constructed. The insulated chamber measured 14 x 14 x 14 inches.

A copper-constantan thermopile with 24 junctions was used. Twelve junctions were in the grain and 12 in the air in the chamber. The ends of the thermopile were connected to a Leeds and Northrup No. 2500 galvanometer with a sensitivity of 0.336 u A/mm. The light source was a small bulb enclosed in a cardboard carton with a 1/2 x 1/4 inch open-

ing and operated from a 6-volt transformer. The photoelectric cell was in a vertical position and enclosed in a tin casing with a horizontal slit about 2 x 1/2 inches. The current from the photoelectric cell, after being increased by a two-tube amplifier, operated a sensitive relay which in turn operated a heavier relay. The second relay completed the circuit to the heating coils in the bottom of the chamber. The temperature of the room was controlled by a thermostat set at 25°C. plus or minus about 1°C.

A one-quart commercial Dewar flask was used as a container for the samples. The flask was fitted with a large rubber stopper through which the thermopile and aeration tubes were inserted. Another opening in the stopper, large enough to hold the funnel used for transferring the grain to the flask, was closed with a small one-holed stopper through which a thermometer was inserted into the grain.

The sample was aerated by forcing air through a metal tube, with slit-like perforations in the bottom half, which extended almost to the bottom of the flask. The air escaped from the top of the flask into the chamber through a short tube. The air pressure was maintained by siphoning water into a sealed 15-litre glass jar from which the air displaced could escape only through an opening at the top connected to the aeration tube. By means of a capillary tube and an adjustable pinch-cock the amount of water siphoned into the jar and thus the amount of air forced

into the grain, was controlled at about 2 litres per day. The humidity of the air entering the grain was regulated by passing the air through a sulphuric acid solution of predetermined strength in two gas washing bottles, one outside the chamber and one inside. The concentration of acid used was determined from tables of concentration of acid and relative humidity of air by Wilson (20) and relative humidity of air and moisture of wheat by Coleman and Fellows (4). A 27% solution of sulphuric acid was used. This gave a relative humidity of about 80% which is equivalent to a grain moisture of about 20%. Graphs made from Wilson's (20) tables showed that at this concentration of acid, the relative humidity changed only from 80% at 25°C. to 81% at 50°C.

Temperature readings were obtained from thermometers graduated in tenths in the grain, in the air in the chamber, and in a flask of water in the chamber. The latter thermometer was added for the later experiments as a means of ensuring that the rise in temperature of the grain was not caused by external heating. If the temperature of the grain and the water were the same at the beginning and if the temperature of the water lagged behind that of the grain during an experiment, it could be accepted that the rise in temperature was due to self-heating.

The temperature control mechanism was standardized with water at about 45°C. in the Dewar flask. The photo-

electric cell and the mirror on the galvanometer were adjusted so that the temperature of the water in the Dewar flask dropped 0.1°C. to 0.2°C. per day. This slight drop in temperature would ensure that during an experiment the grain was not being heated artificially. It would mean, however, that the temperature rise during the experiment did not represent the total heat produced. In later experiments the apparatus was standardized at between 32°C. and 35°C., since there was some possibility that the grain might be heated externally at low temperatures when the standardization was made at the higher temperatures.

When the apparatus was standardized, the water in the Dewar flask was replaced by the previously conditioned sample of grain, care being taken to make certain that the thermopile was replaced in the same position as it was during standardization. When the temperature began to drop, after reaching a maximum, the grain was removed and the Dewar flask filled with water at the final temperature of the grain. A check was made to determine whether the water temperature was dropping. If so, the rise in temperature of the grain represented at least the minimum rise resulting from self-heating.

Procedure for a Typical Experiment

When the adiabatic equipment had been standardized, 600 gm. of the 700 gm. moisture conditioned sample were

transferred to another sterile sealer, after mixing, and tempered in a 28°C. incubator for 24 hours or a 32°C. incubator for 12 hours. The remaining 100 gm. was kept in the refrigerator and was used for plate counts, moisture determinations and germination tests. The Dewar flask and stopper, with the thermopile and aeration tubes, were sprayed with a disinfectant, rinsed in sterile water and dried and tempered at 32°C. for about 2 hours. The stopper was then placed in the Dewar flask, the 600-gm. sample poured through a sterile funnel into the flask, the flask placed in the chamber, the chamber closed, and the control unit connected. Daily records were kept of temperatures of the grain, the water, and the air, and the amount of air replaced, until the maximum temperature of the grain was reached. After heating, the control mechanism was disconnected, the Dewar flask removed and the grain transferred to a sterile sealer. Plate counts, identification studies, moisture determinations and germination tests were carried out as soon as possible. In the meantime, the heated sample was kept in a 50°C. incubator until the results were complete.

EXPERIMENTAL

Trial 1

A sample of Thatcher 47 was moisture conditioned from a normal of 9.40% to 18.30%. Ninety-nine out of 100 seeds tested, germinated. The sample, when placed in the adia-