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

by

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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-

batic chamber on July 23, 1948, was at 26.6°C. The temperature started to rise on the first day and reached 51.0°C. in 15 days, where it remained constant until removed 2 days later. Contrary to expectation, the dilutions used for plate counts made on the day of removal were too high, which meant that it was not possible to estimate numbers of bacteria and fungi on the heated sample. To obviate this loss of plate-count data in subsequent trials, each sample was held at 50°C. until results were complete. The moisture content of the heated sample was 20.20%; and germination 0%. The temperature control mechanism was functioning satisfactorily when tested after the sample was removed.

Trial 2

A moisture conditioned sample of Marquis 47 at 26.5°C. was placed in the adiabatic chamber on August 18, 1948. The temperature rose 0.8°C. the first day and reached 47.0°C. in 20 days. The temperature remained at 47.0°C. until the 24th day, dropping to 41.1°C. by the 26th day when it was removed. This rise in temperature is shown graphically in Fig. 1. Bacterial and fungal counts, moisture determinations and germination data, both before and after heating, are shown in Table 6. The fungi on the heated sample consisted of about 50% *Penicillium melinii* Thom and 50% *P. viride-dorsum* Biourge. A comparison of the occurrence of these species before and after heating is shown in

Fig. 2. The temperature control mechanism was operating properly when tested at the end of the trial.

Trial 3

A sample of Marquis 47 (germination 92% and moisture 18.64%) was placed in the adiabatic chamber on December 30, 1948. A rapid rise in temperature, from 26.0°C. to 43.0°C. in 5 days, aroused suspicion that the grain was being heated externally. However, when the grain was removed and a check made on the apparatus, it was found to be operating properly. The number of bacteria on the sample had been reduced from 190,000 per gm. to 2,000 per gm., while the fungi had increased from 200 per gm. to 1,700 per gm. Aspergillus fumigatus Fesenius made up 75% of the fungi found on the sample after self-heating. The moisture content was 18.80% and germination 8%.

Trial 4

A moisture conditioned sample of Thatcher 47 was placed in the adiabatic chamber on December 20, 1949, after being tempered to 32°C. The temperature of the sample rose to 40.2°C. the first day and continued to rise rapidly, except on the 5th day, until it reached 57.1°C. on the 9th day. The sample was removed on the 10th day after the temperature had dropped to 56.8°C. When the temperature

control mechanism was checked it was found that the water was being heated. Therefore, the sample had probably been heated externally. However, from the data on temperature readings during the trial it was obvious that most of the heat produced was due to self-heating. The number of bacteria and fungi on the grain before and after heating, and the moisture content and germination before and after heating are shown in Table 6. The relatively high bacterial count was not obtained in any of the other trials. The fungi consisted of about 50% Aspergillus fumigatus and 50% Mucor sp. at incubation temperatures of 50°C. and 37.5°C. At 25°C., A. fumigatus accounted for only 14% of the total count. The rise in temperature is presented in Fig. 1; and the change in numbers of A. fumigatus and the Mucor sp. before and after heating in Fig. 2.

Trial 5

A sample of Thatcher 49, moisture 21.77%, germination 99%, was placed in the chamber January 21, 1950. It rose from 30.3°C. to 31.5°C. in the first day and to 45.9 in 15 days. A power failure caused the temperature to drop slightly on the 16th day after which it rose to 47.1°C. on the 20th day. While the trial was being continued to make sure that the maximum temperature had been reached, one of the relays stuck and the grain was heated externally. At

the time of discovery the temperature of the grain was 75°C. and probably had been higher than this since a safety fuse had been melted. The grain was removed immediately and the relay fixed. A standardization check showed that the adjusment was still correct, indicating that the sample had not been externally heated before the relay stuck. The temperature rise is presented in Fig. 1 and the data recorded during the heating in Table 5. In spite of excessive heat at the end of the trial, the fungal count was quite high. It consisted entirely of a thermophilic species of Mucor which had been previously found in Trial 3. A comparison of the number of this species on the sample before and after heating is shown in Fig. 2 and the incidence of certain fungi on the wheat before heating in Table 4.

Trial 6

Another sample of the same wheat was inoculated with Aspergillus fumigatus by conditioning the sample with water in which the conidiawere suspended. Data on counts, moisture and germination before heating are shown in Table 6. From an initial temperature of 30.6°C., the temperature rose rapidly in 5 days to 50.4°C. On the 6th day the sensitive relay ceased to function properly and the grain cooled to 38.8°C. The relay was repaired and heating continued. The temperature reached 50.8°C. on the 8th day. However,

the next morning the temperature was down to 46.9°C. The safety fuse, in the circuit leading to the heating coils, had melted. A new safety fuse was installed and the trial continued. The temperature reached 51.5°C. on the 12th day. The grain, having cooled to 50.6°C., was removed the next day. The rise in temperature for the first 5 days is shown in Fig. 1. Data on counts, moisture and germination are presented in Table 6, and the increase of A. fumigatus during self-heating in Fig. 2. A fumigatus was the only species present at the dilution counted. The temperature control mechanism was operating satisfactorily when tested at the end of the trial.

Table 4. The incidence of certain fungi on a sample of Thatcher wheat before heating.

(Total on 6 plates at a 1:10 dilution)

	25°C.	37°C.	50°C.
Penicillium spp.	36	36	
Scopulariopsis spp.	34		
Alternaria spp.	12		
Aspergillus spp.	9	13	6
Hormodendrum spp.	8		
Cladosporium spp.	5		
Torula spp.	3		
Mucor spp.	2	1	
Others	31	2	1
Total	140	52	7

Table 5. Data recorded during a typical self-heating trial (Trial 5 - January 21, 1950)

Day	Time	Grain Temp.	Water Temp.	Air Temp.	Room Temp.	Water Level*
0	2.30 p.m.	30.3	28.6	31.8	25.0	1.5
1	11.15 p.m.	31.5	31.6	32.4	26.0	3.0
2	9.30 a.m.	31.8	31.8	32.9	24.0	4.0
3	9.45 a.m.	32.1	32.1	33.0	25.0	5.0
4	9.50 a.m.	33.3	33.1	34.3	24.0	6.0
5	9.20 a.m.	34.3	33.9	35.3	23.5	8.0
6	9.05 a.m.	34.5	34.0	35.3	23.5	10.0
7	11.50 a.m.	35.9	35.2	36.8	23.5	12.0
8	-	-	-	-	-	-
9	9.10 a.m.	39.2	38.1	39.7	25.0	15.0 0.0
10	9.20 a.m.	40.8	39.5	41.2	25.0	2.5
11	9.15 a.m.	42.3	41.0	42.8	25.0	5.0
12	9.15 a.m.	43.7	42.2	44.0	23.5	7.0
13	9.15 a.m.	44.6	43.0	44.7	24.0	8.5
14	11.50 a.m.	45.2	43.6	45.4	24.0	10.0
15	9.35 p.m./	45.9	43.0	39.7	24.0	13.5
16	9.10 a.m.	45.7	44.1	45.9	26.0	14.5 0.0
17	9.20 a.m.	46.1	44.5	46.3	24.0	1.0
18	9.35 a.m.	46.5	44.8	46.7	26.0	3.0
19	9.20 a.m.	47.0	45.3	47.1	25.0	5.0
20	9.15 a.m.	47.1	45.4	47.2	25.0	7.0
21	9.35 a.m.	47.0	45.2	47.1	25.0	8.5
22	-	-	-	-	-	-
23	9.35 a.m.	75.0	--	--	--	--

* Water level = level of water in litres siphoned into the jar from which air was forced for aeration.

/ The power was off from 8.45 p.m until 9.35 p.m.

Table 6. Pertinent data on self-heated wheat.

Trial	Sample	Heated	Fungi per gm. 25°	Fungi per gm. 37°	Fungi per gm. 50°	Bacteria per gm. 25°	Bacteria per gm. 37°	Bacteria per gm. 50°	Germination	Moisture
2	Marquis 47	before	180	>10		19.4×10^4	26,000	>10	96%	19.17
		after	46,000	37,000		>1,000	>100	>10	0%	19.87
4	Thatcher 47	before	>10	>10	>10	90,000	17,000	10	92%	23.65
		after	22×10^4	25×10^4	6,100	61,000	44,000	11,500	0%	25.23
5	Thatcher 49	before	30	10	>10	370	100	>10	100%	21.77
		after	46×10^4	$<10 \times 10^5$	$<20 \times 10^4$	>100	>100	>10	0%	21.74
6	Thatcher 49 (inoculated)	before	300,000	65,000	40,000	180	>10	>10	100%	22.85
		after	26×10^6	18×10^6	25×10^6	>100	>100	>100	0%	23.11

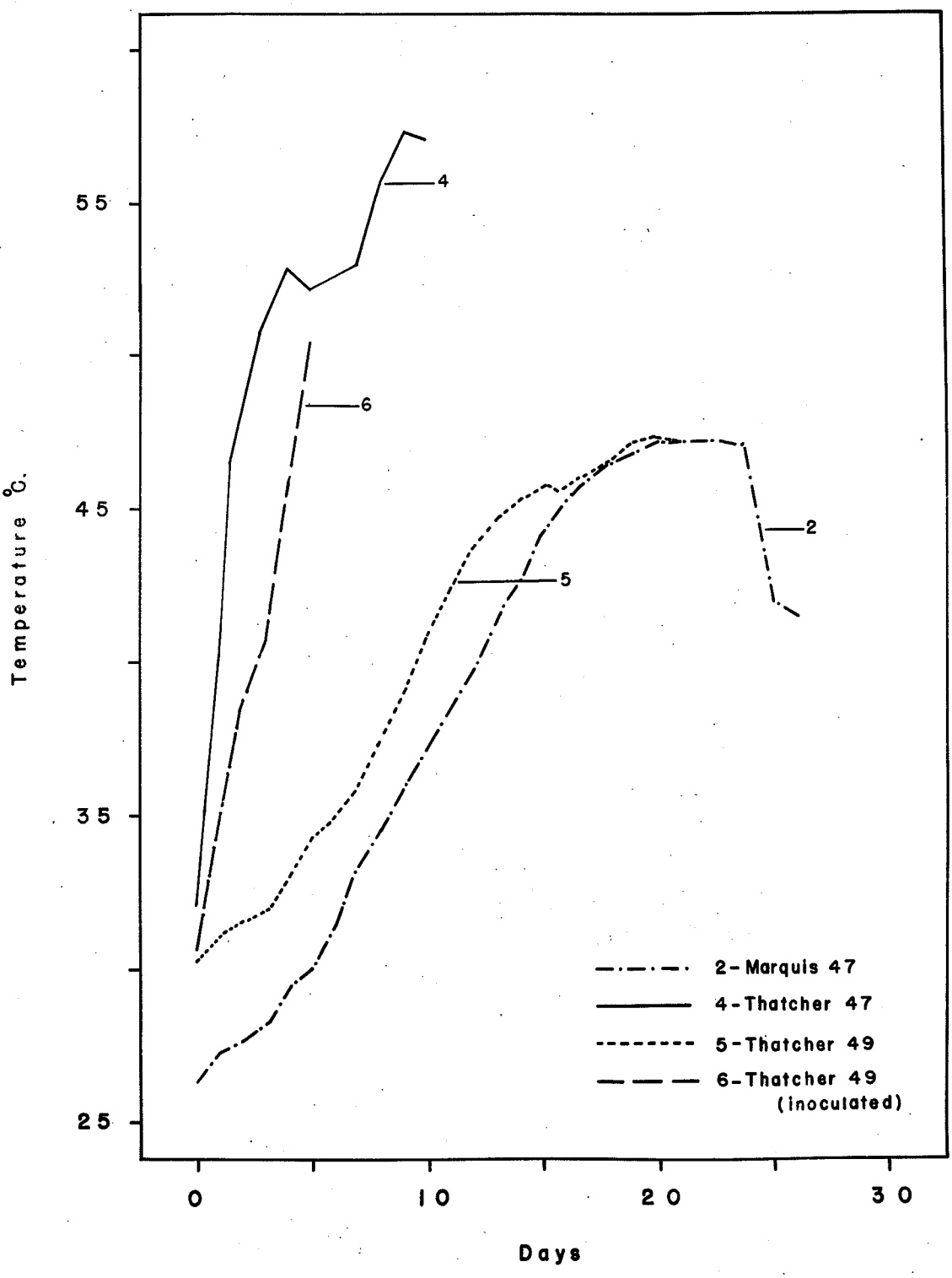

FIG. 1.- Rises in Temperature Recorded During Self-heating Trials.

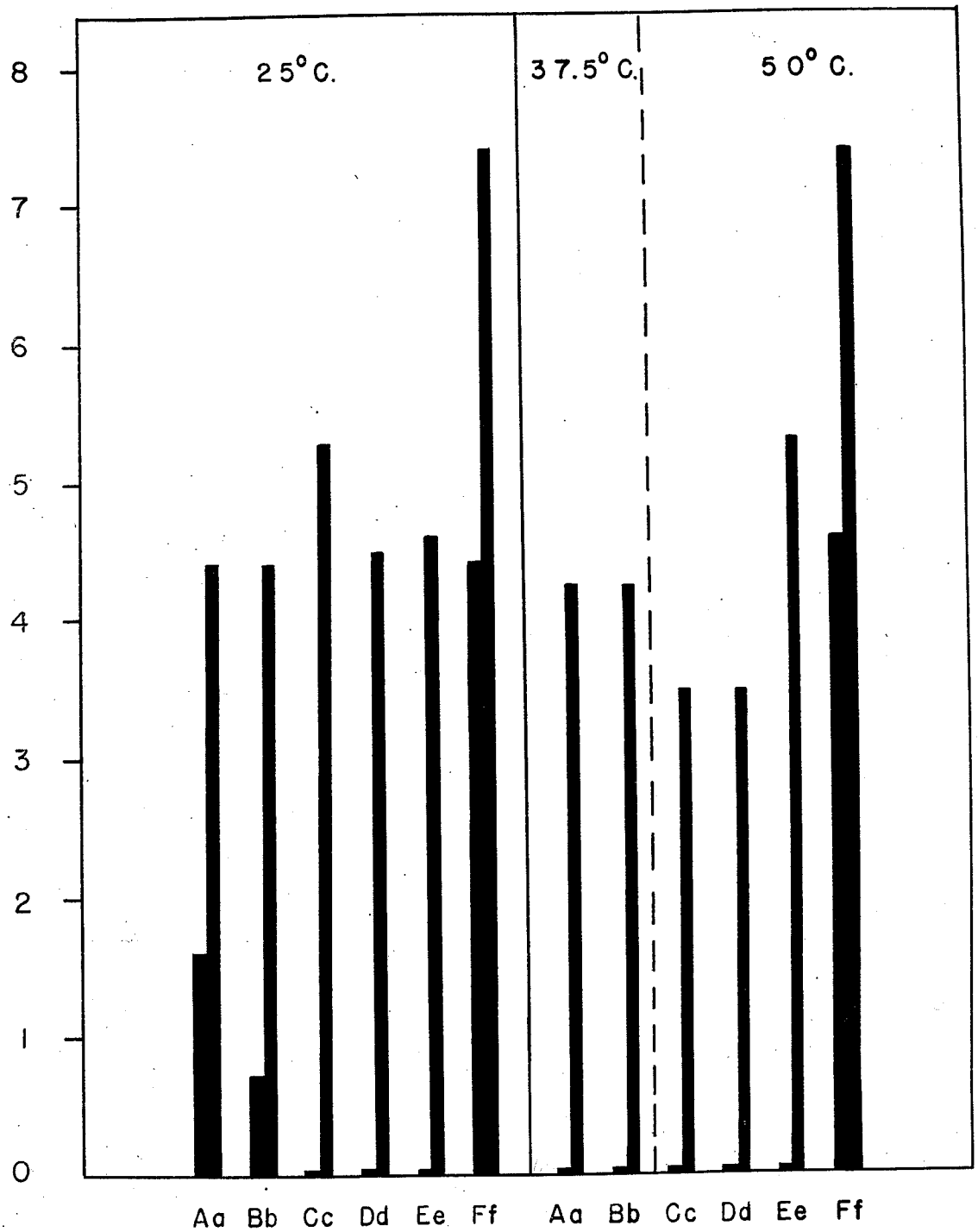

FIG.2- Effect of Self-heating on Numbers of Certain Fungi on Wheat, Expressed as Logarithms.

Aa = Penicillium melinii on Marquis 47
Bb = P. viride-dorsum on same sample
Cc = Mucor sp. on Thatcher 47
Dd = Aspergillus fumigatus on same sample
Ee = Mucor sp. on Thatcher 49
Ff = A. fumigatus on Thatcher 49 (inoculated)

Capital letter designates before heating.

DISCUSSION

In all experiments self-heating was accompanied by an increase in one or two species of fungi. It has been shown (10) that, above 14%, differences in the amount of moisture determines the species of fungi that grow on grain. This may have been the reason for the occurrence of different species of fungi on the heated samples. The species of fungi which predominates during self-heating, as well as the number present, appear to influence the amount of heat produced. The loss in viability of the wheat was probably due both to the activity of fungi and to the effect of heat. Evidence that both reduce the viability of grain has been shown by Swanson (19) and Bakke and Noecker (1).

Temperature rises recorded undoubtedly did not represent the total heat produced. With the apparatus standardized to a drop of 0.1°C. per day at least this much heat was lost during the trials. Thus a sample remaining in the chamber for 20 days would have lost 2°C. and the final reading should have been 2°C. higher. This heat loss probably would be increased due to the fact that when the apparatus was standardized at 33°C., the drop in temperature at 50°C. probably would have been greater than 0.1°C. per day.

An ideal experiment would include standardization, a trial on a sample, a check on the standardization, a

trial on a control sample, and a recheck on standardization. Such an experiment would require about 10 week's continuous operation without adjustment of the temperature control mechanism. So far this has not been accomplished. Some of the difficulties encountered and the remedies used to overcome them follow. It was found that a slight change in the position of the thermopile produced a change in temperature fall at a given setting. This was overcome by fixing the position of the thermopile in the stopper and marking the stopper and the Dewar flask so that the equipment could be assembled and placed in exactly the same position each time. A piece of masonite was placed in the chamber beneath the thermopile to create a "dead air space" around the thermopile where temperature fluctuations probably were at a minimum. Occasionally, a power failure produced sufficient cooling of the air in the chamber to cause the beam of light to swing past the photoelectric cell. An attempt to remedy this was made by installing a stop-block behind the mirror of the galvanometer, and placing a reflector in such a position that when the mirror rested against the stop, the beam of light was reflected back on the photoelectric cell. However, long cooling caused the mirror to pivot on the stop-block. Another stop placed in front of the mirror would prevent this. Also, placing the photoelectric cell in a horizontal position would permit the beam of light to swing over a much wider range. Several failures of the equipment were due to

faulty operation of the sensitive relay. At times the contacts became corroded and fused together, thus causing continuous heating. In these cases the contacts were separated and cleaned. At other times the points of the contacts burned away and proper contact was not made, resulting in cooling of the grain. To remedy this the arms of the relay were bent closer together. The installation of a mercury relay would probably obviate failures due to these causes. Replacement of the small bulb used as the light source invariably necessitated re-standardization, but fortunately, this was not a frequent occurrence.

The problems referred to above and the consequent interference with the normal procedure were responsible for several incomplete trials and the loss of pertinent data. To add to this, standardization of the equipment proved tedious and time-consuming; sometimes as long as two weeks; and this had to be repeated after each failure.

A better understanding of the changes in the microflora of wheat during self-heating probably could be obtained by replicating the experiment a number of times on samples from one source and stopping after different periods of heating in the various trials. A trend in changes in the numbers and types of organisms during heating could then be determined.

SUMMARY

1. An experiment was conducted to determine the relationship between the microflora of wheat and self-heating. Each sample was tested for bacteria, for fungi, for germination and for moisture. It was then moisture-conditioned to a predetermined level and placed in an adiabatic chamber standardized to hold the temperature to a drop of not less than 0.1°C. per day. About 2 litres of air at a humidity in equilibrium with the moisture of the wheat was passed through the sample each day. Daily records of pertinent data were kept. At the cessation of self-heating, the tests used on the unheated sample were repeated. The experiment was replicated with samples of Marquis and Thatcher wheat originating from Melfort, Saskatchewan, 1947, and Lethbridge, Alberta, 1949, and provided by the Dominion Laboratory of Cereal Breeding.

2. The temperature of the samples rose in all trials, with the maximum temperature ranging between 47.0°C. and 51.5°C.

3. The number of fungi, plated on Czapek's agar and on malt agar and incubated at 25°C., 37.5°C., and 50°C., increased during self-heating; while the number of bacteria, plated on nutrient agar and incubated at the same temperatures decreased to insignificance.

In each trial the fungi on the heated sample consisted of only one or two species.

4. Penicillium melinii and P. viride-dorsum were found on one heated sample, a Mucor sp. and Aspergillus fumigatus on another, Mucor sp. only, on two and A. fumigatus only, on two. For the last trial, the sample was inoculated with A. fumigatus at the time of moisture conditioning.

5. Germination was reduced from 96% or more before heating to 0% in all trials except the one that was terminated at 5 days.

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