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Biological, physical and chemical changes in stored wheat

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Abstract

Six lots of wheat from six farmers' bins in Manitoba were adjusted to a range of 5 moisture contents, held at 6 temperatures and sampled at 6 times during storage. After sampling, seeds were surface sterilized with mercuric chloride and subsamples plated on Czapek's agar, on filter paper moistened with water, or on filter paper moistened with a 7.5% sodium chloride solution. The microflora on the seed was determined, germination counts were made, and the seed was examined by government inspectors for its condition and grade. A total of 1 192 samples were examined. In addition, 180 samples were subjected to a fat acidity test. Deterioration for each combination of temperature and moisture, the fungi involved, and the consequent effect on condition, grade and fatty acid content were observed. The effect of high moisture content (>20%) and low temperatures (3–10 °C) on infection of seed by *Penicillium* and the consequent effect on germination, condition, grade and fatty acid value are stressed. The interrelationship among fungi, and among fungi and temperature, moisture, storage time, fat acidity values and germination are indicated by correlation coefficient matrices.

Introduction

In Western Canada the wheat crop is cut and windrowed in August and September when the moisture content (MC) of seed is below 35% wet mass basis (6). Under normal conditions, the crop is then combined about 1 week later (when the seed has less than 14.5% MC) (13). Under poor drying conditions or for management reasons, farmers harvest the grain at higher moisture contents and then mechanically dry it. The problems of grain drying and storage in Canada have been reviewed by Muir (13). The average amount of grain still in storage in Canada at the beginning of a new harvest on August 1st during 1969–78 was 21.4 million tonnes of wheat, oats, barley, rye, flax and rapeseed (3); 60% of this grain was on farms and 24% in primary elevators (13). The call for increased production, especially wheat, in 1981, the difficulty of getting the crop to market, and new legislation aimed at getting the farmer to store grain on the farm stress the importance of research on grain storage. The microflora of stored grain and the factors related to spoilage have been reviewed by Semeniuk (15), Trisvyatskii (22), Christensen & Kaufmann (4), Wallace (25), and the ecology of fungi on stored grain by Pelhate (14) and Sinha (18). The habits and characteristics of each fungus are well-known, and the conditions that permit or promote the growth of each fungus have been established (5), but an integrated knowledge of microflora and associated abiotic and biotic variables is also considered essential for proper management and long-term planning of postharvest grain storage systems (18).

The purpose of this report is to determine the effect of abiotic variables (moisture content, temperature and storage time) on the biotic variables (the seed and its microflora) which cause deterioration, as indicated by seed germination, condition of the grain, its grade, and free fatty acid values.

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Methods and materials

Six lots of 25 kg of hard, red spring wheat were collected in December 1976 from farm granaries near Winnipeg, Manitoba and stored at 15 °C (Table 1). Each seed lot was cleaned to remove dockage and broken seeds; the seed was then examined for embryo exposure (7). Each seed lot was conditioned to 5 moisture levels, stored at 3 °C for 3 days, and shaken frequently to obtain a uniform moisture content. About 3 kg of seed from each moisture level was placed in six 3.6-l jars which were stored at 3, 10, 15, 21, 29 and 40 °C (all ±1 °C) and 360 g subsamples were removed on 6 dates (Table 2). Storage periods for each temperature and moisture content combination were selected on the assumption that the grain would deteriorate and drop in grade midway through the storage period. The total number of samples for the main test was 1 116; 6 seed lots × 5 moisture contents × 6 temperatures × 6 dates + 36 controls. The controls represent the 'zero' time factor and were made up as follows: the six original samples before the water was added plus the 30 seed lots × moisture combinations after the grain had been incubated at 3 °C for 3 days, i.e. at the time the grain was placed in jars.

About 300 g portions of all subsamples were sent to the Grain Inspection Division of the Canadian Grain Commission, Winnipeg, where they were officially graded and their condition noted. Another 60 g portion was surface sterilized by immersion for 3 minutes in a solution containing 1 part 95% ethyl alcohol and 3 parts of a 0.1% aqueous solution of mercuric chloride and then rinsed twice with sterile water (10). Three techniques were concurrently used for fungal isolation and enumeration: (a) the FP technique: 4 plates of 25 seeds on a #3 Whatman filter paper moistened with 4.5 ml sterile water; (b) the SFP technique: 2 plates of 25 seeds on similar fil-

Table 1. Physical, chemical and biological condition of the 6 original lots of hard red spring wheat used in the storage experiment.

Seed condition	Lot No.					
	1	2	3	4	5	6
Before cleaning						
Dockage (%, weight)						
a. Broken wheat	9.2	7.6	13.9	4.0	2.4	2.4
b. Weeds, chaff, etc.	4.1	4.1	6.7	2.0	3.6	8.6
After cleaning						
Grade (CW)	1	1	1	2	2	1
Condition			All 'cool sweet'			
Embryo exposure (%)	19	14	11	2	4	30
Moisture content (%)	12.1	12.2	12.6	13.3	12.7	12.6
Fat acidity value[1] (FAV)	7.6	7.2	7.4	6.9	8.2	8.5
Germination (%) FP	98	98	98	99	96	96
Aspergillus flavus (%) FP	3	0	0	0	0	0
Penicillium spp. (%) FP	11	12	0	0	0	0
Alternaria (%) CZ	74	54	74	94	80	82

[1] Expressed in mg . KOH/100 g of dry seed.

Table 2. Storage period (days) between sampling dates of wheat lots.

Storage temperature °C	Moisture contents (%, wet mass basis)				
	12.7–12.9	14.4–15.2	15.9–16.6	17.3–18.5	21.3–22.2
3	28–31	28–31	28–31	28–31	20
10	28–31	28–31	28–31	20	10
15	28–31	28–31	21	10	3
21	28–31	22	12	6	2
29	10	7	5	4	1
40	4	3	3	2	1

ter paper moistened with 4.5 ml of 7.5% sodium chloride solution; and (c) the CZA technique: 5 plates of 10 seeds on Czapek's solution agar (12). All plates were stacked and enclosed in plastic bags to prevent drying and stored for 1 week at 21 ± 1 °C before reading. Germination counts were made and the fungal flora of each seed were recorded using a stereo microscope (25, 50 or 100×). On the final sampling date, the moisture content (wet basis) was determined by the ASAE oven dry method S352 (2), and the fat acidity values (FAV) by the AACC General Method 02-01 (1).

On 4 April 1977 a supplementary test was initiated using seed held at 15 °C from original lots 1 and 4 (Table 1), and conditioned to initial moisture contents of 16.5, 18.0 and 22%. These jars were incubated at 31 and 40 °C for 3–12 days, then transferred to 21 °C ± 1 °C. Samples were taken at several periods (1–12 days) and subjected to the same tests as in the main experiment; a total of 76 samples were examined.

Table 3. Effect of enumeration technique on germination and frequency of occurrence of fungi. Means of 1192 samples.

	Filter paper (FP) %	Salt filter paper (SFP) %	Czapek (CZA) %
Germination	90.14 (0)[1]	0.00 (1192)	89.44 (0)
Fungi (preharvest)			
Alternaria	2.64 (659)	2.55 (731)	58.18 (16)
Cladosporium	0.28 (958)	0.16 (1117)	0.14 (1116)
Cochliobolus	0.41 (870)	0.01 (1191)	1.09 (761)
Fungi (postharvest)			
Absidia	0.04 (1181)	0.05 (1181)	0.04 (1182)
Aspergillus flavus	5.68 (610)	2.68 (862)	5.09 (742)
A. glaucus group	0.58 (1100)	4.36 (905)	0.94 (1092)
A. niger	0.38 (1085)	0.06 (1178)	0.53 (1113)
A. versicolor	0.06 (1184)	0.07 (1167)	0.00 (1192)
A. ustus	2.42 (735)	1.75 (939)	3.14 (822)
Mucor	0.73 (753)	0.18 (1111)	1.17 (852)
Penicillium	13.17 (342)	8.13 (673)	14.54 (454)
Rhizopus	1.51 (663)	0.18 (1120)	1.01 (881)
Bacteria	0.06 (1136)	0.03 (1181)	0.79 (928)

[1] Figures in brackets indicate number of 'zero cases'.

Results

The biological, physical and chemical characteristics of the six lots of wheat at the time they were collected from the bins are shown in Table 1. Considering condition, grade, germination and fat acidity value (FAV), all lots were 'sound', even though some *A. flavus* and *Penicillium* infection occurred in lot 1 and some of the latter in lot 2.

Surface sterilization

Surface sterilization dramatically reduced *Alternaria* from the seed surface (Table 3). This fungus occurred on 58% of the seeds plated on CZA, but only slightly over 2% on filter paper to which water or NaCl solution had been added. This difference between the CZA and FP does not occur when the seed is not surface sterilized (26). Other preharvest fungi were not similarly affected.

Fungal enumeration technique

The comparison of fungal enumeration techniques is based on 1 192 samples from the main test, the controls and the supplementary test (Table 3). With the exception of *Alternaria,* the frequencies of occurrence of each fungus are similar for both the FP and CZA techniques. The frequencies of all fungi on SFP, except the *A. glaucus* gr., are equal to or slightly lower than frequencies of occurrence of the same fungi on other media. *A. glaucus* group always occur more on SFP than on the other media. The data presented contain many 'zero's' since no deterioration occurred under 'dry' conditions; only *Penicillium* occurred under 'cold' conditions.

The data for CZA were presented in the remaining tables of results because *Alternaria* gave the best results on CZA, and other fungi grew well on this medium (*A. glaucus* gr. excepted); the data for *A. glaucus* gr. are, however, those for SFP. Furthermore, to remove most of the 'zero's' only the data for the last sampling date are given unless otherwise stated. The storage time is included in many tables because the time factor varies from 6 to 182 days (Table 2).

Seed germination

Germinability of seed is used as an indicator of deterioration (Table 4). All original seed had 96% germination or higher. Seed samples germinating 85% or higher were considered 'sound' and anything below that as 'deteriorated'. Table 4 has 3 sec-

Table 4. Effect of moisture content and temperature on seed germination odour and grade. Results are for 6 lots of wheat, except where otherwise footnoted.

Moisture content (%)	Temperature (°C)	Storage time (days)	Germination				
			Mean (%)	Range (%)	Lots over 85% (%)	Odour[1] (%)	Degraded[2] (%)
(a) Germination over 85%.							
12.7–12.9	3–21[3]	182	97.5	92–100	100	0	0
12.7–12.9	29	60	98.0	94–100	100	0	0
12.7–12.9	40	24	98.0	96–100	100	0	0
14.4–15.2	3–15[4]	182	95.2	90–100	100	0	0
14.4–15.2	21	134	96.7	86–100	100	0	0
15.9–16.6	3–10[5]	182	94.7	88–100	100	75	17
15.9–16.6	15	126	98.3	94–100	100	100	17
17.3–18.5	3	182	98.7	98–100	100	100	17
(b) Germination above and below 85%.							
14.4–15.2	29	42	92.7	78–100	83	100	0
14.4–15.2	40	18	80.7	32– 98	67	100	0
15.9–16.6	21	72	90.0	78– 98	83	100	17
15.9–16.6	29	30	81.3	60–100	50	100	0
17.3–18.5	10	120	90.3	68–100	83	100	17
17.3–18.5	15	60	78.7	72– 90	33	100	17
17.3–18.5	21	36	65.3	52– 88	17	100	17
21.3–22.2	15	18	69.7	44– 92	50	100	17
21.3–22.2	21	12	69.7	34– 86	17	100	0
21.3–22.2	29	6	58.7	50– 92	17	100	0
(c) Germination below 85%.							
15.9–16.6	40	18	50.7	22– 72	0	100	0
17.3–18.5	29	24	54.0	34– 70	0	100	17
17.3–18.5	40	12	55.3	40– 84	0	100	17
21.3–22.3	3	120	44.0	28– 58	0	100	100
21.3–22.3	10	60	44.3	28– 72	0	100	50
21.3–22.3	40	6	50.7	16– 82	0	100	0

[1] Percentage of lots on which Canadian Grain Commission Inspectors provided sensory judgements as having a fermented, musty or sour odour at or before the indicated storage time.

[2] Percentage of lot which Canadian Grain Commission Inspectors indicate have undergone a reduction in grade at or before storage time indicated.

[3] 24 lots. [4] 18 lots. [5] 12 lots.

tions which represent conditions under which the effect of moisture and temperature was: (a) nil, (b) inconsistent, and (c) seed germination lower than 85%. Different reactions of seed lots to the moisture-temperature combinations in group (b) caused the inconsistency.

Seed lot

Of 30 moisture and temperature combinations only 7 caused deterioration of lot 4 while 15 caused deterioration of lot 1 (Table 5). The principal preharvest fungus was *Alternaria* which varied from 39.0 to 72.1% infection in sound grain. *Cochliobolus* was present in all lots but never exceeded 1.9% infection on sound seed. *Penicillium* was common in the deteriorated seed of all lots and was, therefore, not responsible for differences between seed lots. *A. glaucus* gr. occurred less frequently but also tended to be of a low level for all lots. Neither of these fungi were probably the cause of the differences in deterioration among the lots. Infection by *A. flavus* was higher in those lots undergoing the most deterioration as indicated by lowered seed germination. For lots 1 and 2, *A. flavus* infected 23% of the seeds while 48% of the samples had seed germination less than 85%. For lots 3 and 6, *A. flavus* infection was down to 16% while the percent

Table 5. Effect of seed lot on germination (%) and frequency of occurrence of fungi (%) on six wheat lots incubated at different combinations of temperature and moisture.

Deteriorated[1] samples	Seed[2] germination	Lot No.						
		1	2	3	4	5	6	Mean
		50	47	40	23	27	33	37
Germination	s	99	96	95	96	96	96	96
	d	43	47	52	68	67	66	57
Alternaria	s	48	39	52	72	61	59	55
	d	17	18	23	53	36	22	28
Cochliobolus	s	0	0	1	2	2	2	1
	d	1	0	0	1	1	2	1
Penicillium	s	13	10	6	5	4	10	8
	d	41	57	48	45	52	49	42
A. flavus	s	9	5	1	1	0	0	3
	d	26	20	19	2	9	13	15
A. glaucus gr.	s	2	7	2	1	1	0	2
	d	13	22	12	11	13	16	15
A. ustus	s	6	3	1	0	0	0	2
	d	16	1	1	0	0	0	3
A. niger	s	0	0	0	0	0	0	0
	d	1	0	1	0	4	10	3
Rhizopus	s	2	3	1	0	0	0	1
	d	5	3	2	0	0	0	2
Mucor	s	1	2	1	0	0	0	1
	d	5	2	1	0	0	0	2

[1] Percentage of 30 samples of lot with viability less than 85%.
[2] s – means of samples with viability 85% and over.
d – means of samples with viability less than 85%.

deteriorated samples decreased to 37%. Lowest *A. flavus* infection (5%) and percent deteriorated samples (25%) occurred with lots 4 and 5. Therefore, this fungus could have caused the differential response of seed lot deterioration. The appearance of *A. ustus* (Bain) Thom and Church and *A. niger* Van Tieghem can be considered as isolated cases. *Emericella nidulans* (Eidam) Vuillemin, *A. versicolor* Tiraboschi and *A. wentii* Wehmer occurred rarely; *A. candidus* Link was also relatively scarce and considered negligible in this experiment.

Storage time

Based on the seed germination criterion, seed having 12.7–12.9% MC can be stored safely for more than 180 days at 3–21 °C, 60 days at 29 °C, and 24 days at 40 °C (Table 6). Seed with 14.4–15.2% MC cannot be stored safely for more than 21 days at 40 °C. Seed at 15.9–16.6% MC will keep for only 36–48 days at 21 °C and less at higher temperatures. The higher the MC the wider the range of temperatures that cause deterioration, e.g.

Table 6. Storage periods (days) after which seed germination decreases below 85%. When wheat was stored at various combinations of temperature and moisture content.

Moisture content (%)	Temperature (°C)					
	3	10	15	21	29	40
12.7–12.9	180+	180+	180+	180+	60+	24+
14.4–15.2	180+	180+	180+	132+	42+	21–28
15.9–16.6	180+	180+	126+	36–48	15–20	3– 6
17.3–18.5	180+	120+	40–50	12–18	4– 8	4– 6
21.3–22.2	40–60	10–20	6– 9	4– 6	1– 2	1– 2

at 21.3–22.2% MC the samples deteriorated in 1–20 days at temperatures ranging from 10–40 °C.

Interaction of moisture content and temperature on fungi and germination

An increase in MC from 17.3–18.5% to 21.3–22.2% at 3 °C increased *Penicillium* from 5 to 98%, decreased *Alternaria* from 59 to 9%, and germination from 99 to 43% (Table 7). Similar results were obtained at 10 °C. At these low temperatures, MC at 17.3–18.5% is the factor limiting occurrence of *Penicillium* and its consequent inhibiting affect on *Alternaria* and reduction in germination. However, as the temperature rises the limiting effect of *Penicillium* is reduced, e.g. at 10, 15 and 21 °C *Penicillium* infection increases drastically from 3 to 62% with a consequent reduction in *Alternaria* and germination. In contrast, at 21.3 to 22.2% MC and 3, 10 and 15 °C the frequency of occurrence of *Penicillium* is 98, 99 and 86% respectively; the corresponding figures for *Alternaria* are 9, 13 and 44% and for germination are 43, 70 and 70%. Although *Penicillium* appears to be least pathogenic at 15 °C for this MC, this result was probably caused by the shorter storage times. At 21 °C the *A. glaucus* gr. appeared, especially on seed of 15.9–16.6% MC (Table 7). At higher moisture contents this fungus was replaced by *Penicillium*. At 29 °C and 15.9–16.6% MC the *A. glaucus* gr. are the predominant storage fungi, but as the MC increases, these fungi are replaced by *Penicillium* and *A. flavus*. At 40 °C *Penicillium* is no longer a factor (Table 7). At 40 °C the predominant storage fungi change from *A. glaucus* gr. at 14.4–16.6% MC to *A. glaucus* gr. associated with *A. flavus* at 17.3–18.5% MC to *A. flavus* at 21.3–22.2% MC (Table 7).

Except for trace amounts, *E. nidulans* was important in only one sample. It occurred on seed having 22.2% MC and stored at 40 °C. It was found on 19% of the seeds and grew in association with 46% infection by *A. flavus* and 24% infection by *Absidia*. *A. niger* occurred on several samples when the moisture content was 18–22% and at temperatures of 29–40 °C. It was always associated with *A. flavus*. Both *A. niger* and *E. nidulans* appeared on seed after it had become infected by *A. flavus*.

Effect of cooling grain after storage at high temperatures

When seed with 15.9–18.5% MC is stored for

Table 7. Selective action of temperature and moisture on germination and fungus flora grown on wheat stored at various combinations of temperatures and moisture. Means of 6 lots.

Temp. (°C)	MC (%)	Time (Days)	Germ.[1] (%)	*Alt.* (%)	*Pen.* (%)	*A. fl.* (%)	*A. gl.* (%)	*A. us.* (%)
3	17.3–18.5	182	99	59	5	3	2	9
3	21.3–22.2	120	43	9	98	0	0	0
10	17.3–18.5	120	90	51	3	3	3	4
10	21.3–22.2	60	43	13	99	0	0	0
15	17.3–18.5	60	79	44	48	1	7	3
15	21.3–22.2	18	70	44	86	0	0	3
21	15.9–16.6	72	90	69	2	1	20	0
21	17.3–18.5	36	65	28	62	1	11	1
21	21.3–22.2	12	59	29	89	16	0	7
29	15.9–16.6	30	81	54	1	1	11	0
29	17.3–18.5	24	54	29	60	16	28	2
29	21.3–22.2	12	64	42	55	40	1	16
40	14.4–15.2	18	81	4	2	2	5	1
40	15.9–16.6	18	51	26	1	1	47	1
40	17.3–18.5	12	55	31	5	18	47	2
40	21.3–22.2	6	51	21	2	70	4	3

[1] Abbreviations: Germ. = germination,
Alt. = *Alternaria*,
Pen. = *Penicillium*,
A. fl. = *Aspergillus flavus*,
A. gl. = *A. glaucus* gr.,
A. us. = *A. ustus*.

Table 8. Correlation matrix of fungal variables isolated by three techniques (decimal points omitted, based on 1192 samples).

		CZA	FP	SFP
Preharvest fungi:				
Alternaria	- *Cladosporium*	09	–	–
Alternaria	- *Cochliobolus*	22	–	–
Preharvest and postharvest:				
Alternaria	- *Penicillium*	-56	–	–
	- *A. flavus*	-34	–	–
	- *Rhizopus*	-31	–	–
	- *Mucor*	-25	–	–
	- *A. niger*	-24	–	–
	- *A. glaucus gr.*	-18	–	–
	- *Absidia*	-17	–	–
Postharvest:				
A. flavus	- *A. niger*	57	44	24
	- *Absidia*	37	18	26
	- *A. ustus*	30	37	25
	- *Mucor*	25	27	13
	- *Penicillium*	22	22	24
	- *Rhizopus*	18	23	23
Penicillium	- *Rhizopus*	35	21	21
	- *Mucor*	22	21	05
Rhizopus	- *Mucor*	50	23	26
A. glaucus gr.	- *Penicillium*	-04	01	03
	- *A. flavus*	-01	00	13
	- *A. niger*	02	18	03

Correlation coefficients greater than 0.06 are significantly different from 0 ($P < 0.05$) and correlation coefficients greater than 0.08 are significantly different from 0 ($P < 0.01$).

several days at 40 °C, it can become infected with the *A. glaucus* group (Table 7). Growth of these fungi continued after the temperature was lowered to 21 °C with a consequent reduction in germination after 4 to 5 weeks. Seed with 21.3–22.2% MC stored for several days at 40 °C favoured infection by *A. flavus*, and cooling the grain at 21 °C had no effect on this fungus. Lowering the temperature to 21 °C did not result in increased *Penicillium* invasion of the seed already infected by *A. flavus*. In contrast, when the initial temperature was 31 °C for several days before cooling to 21 °C, both *A. flavus* and *Penicillium* developed together.

Interrelationships among fungi stored in wheat

The correlation coefficient matrix of all 1192 samples for each of the fungal enumeration techniques was calculated (Table 8). The correlations for *Alternaria* and other fungi on FP and SFP are omitted because surface sterilization caused unnaturally low levels. Preharvest fungi have positive correlations with each other, and so do most of the postharvest fungi, but preharvest fungi correlated negatively with postharvest fungi. Generally, the correlations are similar for the different enumeration techniques, *Penicillium-Mucor* and *A. glaucus-A. flavus* on SFP and *A. glaucus-A. niger* on FP are possible exceptions. With those exceptions the *A. glaucus* gr. shows little correlation with the other storage fungi.

The correlations of the four principal fungi with temperature, moisture, time and their effect on germination and fat acidity value (FAV) were de-

Table 9. Pooled correlation coefficients of all 6 lots of wheat (CZA except *A. glaucus* gr. on SFP).

Temperature	×	*Alternaria*	-0.493
	×	*A. glaucus* gr.	0.463
	×	*A. flavus*	0.379
	×	*Penicillium*	-0.121
	×	FAV	-0.122
	×	Germination	-0.283
Moisture	×	*Alternaria*	-0.385
	×	*A. glaucus* gr.	0.064
	×	*A. flavus*	0.459
	×	*Penicillium*	0.681
	×	FAV	0.488
	×	Germination	-0.720
Time	×	*Alternaria*	0.589
	×	*A. glaucus* gr.	-0.388
	×	*A. flavus*	-0.378
	×	*Penicillium*	-0.356
	×	FAV	-0.166
	×	Germination	0.595
A. glaucus gr.	×	*A. flavus*	0.066
	×	*Penicillium*	0.052
	×	FAV	0.047
	×	Germination	-0.367
A. flavus	×	*Penicillium*	0.052
	×	FAV	-0.090
	×	Germination	-0.390
Penicillium	×	FAV	0.620
	×	Germination	-0.642
Alternaria	×	*A. glaucus* gr.	-0.152
	×	*A. flavus*	-0.265
	×	*Penicillium*	-0.492
	×	FAV	-0.489
	×	Germination	0.646
FAV	×	Germination	-0.612

Correlation coefficients greater than 0.057 are significantly different from 0 ($P < 0.05$) and greater than 0.074 for ($P < 0.01$).

termined. The homogeneity of these correlation coefficients across the 6 lots was then tested using the chi-square statistic (20). This statistic revealed no significant differences between lots for all correlation coefficients ($P > .01$); the pooled correlation coefficients are therefore presented in Table 9. The effect of time is an artifact imposed by the experimental plan, e.g. seed stored under dry conditions for a long time were infected by *Alternaria* and germinated well; and seed stored under warm, moist conditions for a short time were infected by storage fungi and soon died.

The strongest correlations were as follows:

Alternaria was detrimentally affected by an increase in temperature (-0.493) or moisture (-0.385).

A. glaucus gr. were favoured by higher temperatures (0.463) but an increase in moisture (0.064) had no effect.

Table 10. Effect of temperature, moisture and time on FAV (mg KOH/100 g of dry seed) of wheat stored at different combinations of temperature and moisture.

Temp. (°C)	Time (days)	Lot					
		1	2	3	4	5	6
Moisture content 17.3–18.5%							
3	182	12†	13	15	11	14	16
10	120	25†	14	15	14	16	14
15	60	20†	15	15	12	12	14
21	36	34†	18	16	13	16	16
30	24	31†	28	24	19	24	20
40	12	20†	20	18	13	18	18
Moisture content 21.3–22.2%							
3	120	80†	65†	46†	35†	29†	36†
10	60	72	59	50	42†	21†	41†
15	18	63	37	34	14	–	17
21	12	32	22	30	12	16	20
30	6	15	10	16	10	11	13
40	6	8	10	10	12	12	18

† Samples that were degraded.

Table 11. Characteristics of samples classified according to grade, odour, visible mould and FAV.

	Number of samples	Number of samples visibly mouldy	FAV	Moisture content	Germinations	*Alternaria*	*Aspergillus flavus*	*A. glaucus* gr.	*A. ustus*	*Penicillium*	*Rhizopus*
				%	%	%	%	%	%	%	%
A. *Grade*											
1 CW	105	7	18	16.0	80	40	8	8	3	23	1
2 CW	65	3	14	16.2	87	58	4	6	1	14	0
3 CW and 3 Utility	10	9	42	21.1	51	13	1	0	0	89	2
B. *Odour*											
Cool-sweet	92	0	14	14.1	96	56	2	2	1	6	1
Slightly musty	25	1	15[1]	17.2	79	46	11	12	2	22	1
Musty	56	18	26	19.6	58	27	10	13	1	55	2
Slightly fermented	3	0	16	17.9	93	51	1	0	0	3	1
Slightly sour	3	0	18	14.2	72	14	0	17	0	2	0
Sour	1	0	84	21.9	16	14	70	2	24	2	8
C. *Visible mould*[2]											
1 CW	7	–	38	20.8	48	17	10	19	0	85	3
2 CW	3	–	13[3]	21.9	78	54	27	0	0	43	0
3 CW or 3 Utility	9	–	44	20.9	49	13	0	0	0	98	2
D. *FAV* (mg KOH/100 g dry seed)											
8.4–14.9	93	2	–	15.4	91	54	6	2	2	10	1
15.0–19.9	53	1	–	15.8	82	43	6	10	2	16	1
20.0–29.9	17	5	–	18.4	56	22	11	26	2	52	1
30.0–39.9	7	2	–	20.1	50	20	14	10	7	86	2
40.0–79.7	9	8	–	21.7	37	11	0	0	2	98	4

[1] Mean of 24 samples.

[2] Characteristics of samples having visible mould classified according to grade.

[3] Mean of 2 samples.

Table 12. Storage period (days) required for grain to become musty[1,2] when wheat was stored at various combinations of temperature and moisture content.

Moisture content (%)	Temperature (°C)					
	3	10	15	21	29	40
12.7–12.9	180+	180+	180+	180+	60+	24+
14.4–15.2	180+	180+	180+	132+	21–42+	6–18+
15.9–16.6	30–180+[3]	30–180+[3]	21–126+[3]	12–60	5–25	3–9
17.3–18.5	30– 60[3]	20– 60	10– 20	6–12	4	2–4
21.3–22.2	20– 40	10	3	2	1	1

[1] Determined by Canadian Grain Commission Inspectors.
[2] Including 'fermented' and 'sour'.
[3] Mustiness usually disappeared after 1–3 months storage.

A. flavus was favoured by both high temperature (0.379) and high moisture content (0.459).

Penicillium was favoured by high moistures (0.681) but high temperatures had a slightly negative effect (–0.121).

All storage fungi had a detrimental effect on *Alternaria* (–0.152 to –0.492), but they had no effect on each other, possibly due to their preference for different moisture-temperature combinations. All storage fungi reduced germination.

Fat acidity value

Fat acidity values (FAV) were similar for all seed lots stored with 12.7–15.2% MC. When the MC was 17.3–18.5% and at temperatures 21 °C or higher, there were occasionally some increases in FAV. The most striking results were the high FAV's at high moisture contents, low temperatures and long storage times; this relationship held for all seed lots (Table 10). The correlation matrices (Table 9) indicate that FAV is positively correlated with *Penicillium* (0.620) and moisture (0.488), but negatively with temperature (–0.122). *A. glaucus* gr. (0.047) and *A. flavus* (–0.090) had little effect on FAV. The highest FAV's were due to *Penicillium* infection (Table 11). Although, *A. glaucus* gr. and *A. flavus* were present when the FAV was between 20–40 mg KOH/100 g of dry seed. They were present less frequently when FAV was higher at 40–79.7 mg KOH/100 g. *Rhizopus* may also contribute to FAV.

Grade and condition

A 'musty' condition occurred in wheat containing moisture contents greater then 15.9% (Table 12). In the 15.9 to 18.5% MC range this condition was described as 'very slightly' to 'slightly' musty and at 21.3% it was always called 'musty'. All storage fungi appeared to be associated with a 'musty' condition (Table 11) and all seed showing visible mould were also 'musty'. In contrast 'musty' seed does not necessarily have visible mould.

Lot 1 was degraded 56 times while the other lots were degraded 3 times or less (Table 13). All lots of

Table 13. Effect of seed lot on condition and grade of wheat stored at different combinations of temperature and moisture content.

Seed lot	No. of samples with the condition					
	Cool-sweet[1]	Fermented[1]	Musty[1]	Sour[1]	Visible mould	No. degraded[1]
1	74	7	83	4	7	56
2	87	8	73	6	9	3
3	104	7	61	3	5	2
4	100	1	83	1	7	3
5	81	5	94	6	4	3
6	99	5	99	3	8	2

[1] Based on sensory judgements of Canadian Grain Commission Inspectors.

wheat having 21.3 to 22.2% MC were degraded at 3 °C but only one-half of the lots were degraded at 10 °C within the storage periods of 120 and 160 days respectively (Table 10). At higher temperatures the grain was not degraded within the selected shorter time periods. At an MC of 17.3–18.5% only lot 1 was degraded; but at this moisture content lot 1 was degraded at all temperatures. Although 'visible mould' always was associated with degrading (100% of 13 samples) at 3 °C, this was not true at 10 °C when only 18% of 17 samples were degraded. Although degrading tended to be associated with high FAV's, some samples with high FAV's were not degraded while some samples with low FAV's were degraded (Table 10). The development of off-odours was associated with drops in germination but degrading was not strongly associated with germination (Table 4). The FAV, moisture content, germinability and fungus infection for grades 1 and 2 CW were similar, but for 3 CW and 3 Utility the FAV contents were high, germinability was low and *Penicillium* infection was high (Table 11). In these experiments four original lots of grain graded 1 CW and two 2 CW. The latter had lower FAV's, higher germinability and lower storage fungi. Samples of grain at harvest time can have widely different floras without affecting the grade. These can affect the initial stages of storage, but eventually, as noted by Machacek *et al.* (11), the initial floras become modified in the same direction until all samples are overgrown by the same fungi to the same degree.

Discussion

An $HgCl_2$ solution in ethyl alcohol was used to sterilize seeds because Machacek *et al.* (10) found it satisfactory after using it in an extensive survey of seed-borne fungi. The alcohol was added to facilitate wetting of the crease of the seed. Stawicki *et al.* (19) found that under laboratory conditions, $HgCl_2$ appeared much more effective than sodium hypochlorite. Only internal fungi were of interest in this study. However, an unexpected result was obtained with *Alternaria*. On CZA 58% *Alternaria* was found, on FP and SFP less than 3% (Table 3). On non-surface sterilized seed *Alternaria* on FP equalled that on CZA. Torrow (21) has shown that mercury cannot be removed by simple washing. However, Machacek (9) and later Wallace & Machacek-(24) found that mercurial fungicides diffuse out from seed into potato-dextrose agar. Presumably the resultant diffusion permits *Alternaria* to grow when seed is plated on agar, but not when plated on FP and SFP. There is no indication that the other fungi were affected by $HgCl_2$ surface sterilization. Machacek *et al.* (11) found that *Rhizopus* developed mostly in stored untreated grain and *Penicillium* in grain treated with an organic mercury seed dressing.

Wallace & Sinha (23) and Sinha & Wallace (17) have reported on the microflora of heated grain. In our experiments the grain was held at constant temperatures. All postharvest fungi showed negative correlations with *Alternaria*. In contrast, all the postharvest fungi, except the *A. glaucus* gr., showed positive correlations with each other. *Penicillium* grew under cool-moist conditions, the *A. glaucus* group under warm and relatively dry conditions, and *A. flavus* under warm-moist conditions. Overlapping of fungi under particular conditions seemed to have no effect on individual fungal species. The growth of *Penicillium* in a granary at temperatures of −5 to 8 °C was confirmed by our tests.

Sinha & Wallace (17) have found that in some years hot spots occur frequently on farms and in country elevators in Western Canada. Wallace & Sinha (23) studied 13 bins ranging from normal to heated grain. Moisture content of heated grain ranged from 24.0 to 27.3% and the principal fungi found were *Penicillium* and *A. flavus*. Hot spots can also occur in large bulks, e.g. Sinha *et al.* (16) found them in a bulk of 9 000 t of wheat that had been stored for seven years in an abandoned airplane hangar. When the bulk was examined the temperatures of the grain ranged from −1 to 7 °C and the MC was 12.6 to 16%. The principal fungi were *Penicillium, A. versicolor* and *Streptomyces,* and in zones previously heated *A. candidus* and *Scopulariopsis*. Wallace *et al.* (26) late in November 1959 placed a 27.2 kg lot of damp grain into a bin of wheat. The original 28 °C temperature of this lot rapidly declined to 8 °C by the end of the month and to 3 °C by April 1960 while the MC rose from 18.5 to 23.0%. *Penicillium* increased and germination decreased during this period and later, in May, developed into a hot spot; the hot grain mass cooled to 19 °C by June. During the heating, or immediately afterwards, the grain became heavily infect-

ed with *A. glaucus* gr. During this stage the MC was 17.8%. A principal component analysis of these data (27) emphasizes the importance of time and to a lesser degree moisture on growth of storage fungi and subsequent germination loss.

Mustiness in foods has been associated with *Pseudomonas* and *Achromobacter* in moist foods, and in drier foods with *Actinomyces* and *Streptomyces*. Jensen (8) states, 'We have never observed moulds as a causative agent of true mustiness in foods', but Stawicki *et al.* (19) list 9 *Aspergillus* spp., 11 *Penicillium* spp., *Alternaria, Cephalosporium* and other fungi which produced a musty odour when grown on coarse wheat meal. They also state that odour was most evident when the fungi are in a profuse spore stage. We have no evidence that seed infected with *Alternaria* and possibly with *Penicillium* or *A. flavus* as in lot 1 and stored with 12.7–12.9% MC ever became musty. At higher moisture contents many samples were musty, but few showed 'visible' moulds. If mustiness is associated with actively growing fungi, then all kinds of fungi were involved. However, a few samples of lot 4 wheat produced no fungi when seed was plated and yet 'mustiness' was detected. It must be remembered that all our seed was surface sterilized hence the almost total absence of *Streptomyces*.

Christensen & Kaufman (4) have reviewed the information on fat acidity. They emphasize that fat acidity varies with the species of fungus and probably with strains within a species, and that a given fungus may produce a relatively large quantity of free fatty acids and then consume large portions of them. Our data clearly establish that FAV is correlated with *Penicillium* infection (–0.54 to –0.83) at low temperatures (3–10 °C) and at moisture contents over 20%. In contrast, the *A. flavus* correlation with FAV varied from 0.08 to –0.26. Although *A. flavus* infected over 70% of the seed stored at 40 °C and at 21.3–22.2% MC, there was little change in FAV. Shortage of storage time – only 6 days – might account for the unchanged FAV level. The rise in FAV at 21–30 °C with the 17.3–18.5% MC may have been caused by *Penicillium* but the situation is complicated by the presence of the *A. glaucus* gr. There is no indication that *A. glaucus* gr. increased the FAV, e.g. the range of correlations were –0.02 to 0.16.

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