CERTAIN STUDIES ON THE MICROFLORA OF WHEAT

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INTRODUCTION

Heating damage in stored grain presents an acute economic problem in Canada and the United States today. This has been accentuated by the necessity of storing large quantities of grain, for longer periods of time than was the case in prewar days. In May 31, 1943, the total storage in Canada had risen to 603 million bushels, an increase of 180 million bushels or approximately 43 percent of the storage capacity over 1939 (24). Formerly the view was held that this spoilage is due to increased respiration in wheat of high moisture content (1). In more recent years there has been some doubt as to the validity of this theory. Workers using various grains and grain products have brought forward presumptive evidence that the primary factor in this heat damage may be micro-organic activity. Many investigators (6, 14, 22) attribute the damage to fungal growth, or indicate that fungi are the primary cause of heating, with bacterial infection a contributing factor at higher temperatures; while some (15, 19, 21) merely state more generally that the increased carbon dioxide out-put in damp wheat is due to micro-organic activity. Due to the fact that the presence of bacteria is not readily apparent, the probable part played by bacteria in this spoilage has been given only passing mention.

Without information as to the numbers and types of organisms on normal wheat, it is difficult to assess the value of results obtained from reports on spoiled grain. Therefore much fundamental experimentation is necessary before the role of micro-organisms in heating damage to wheat can be properly evaluated. An investigation of the flora of normal wheat was undertaken as the first step towards a better understanding of the problem. The study was

carried out by the technique of the bacteriologist interested primarily in micro-organisms of spoilage.

HISTORICAL

Kent-Jones and Amos, 1930, (12) carried out preliminary studies on the bacteriology of wheat and flour. Counts of bacteria in six samples of low grade Manitoba wheat, plated in nutrient agar at 37°C. for 48 hours, ranged from 1,260,000 to 8,000,000 per gram. Fifteen samples from other countries gave counts between 8,000 and 219,000 per gram. The count on samples held under normal storage conditions became lower, whereas counts on samples that were conditioned in the laboratory increased. Counts on plates incubated at 20°C. were larger than those at 37°C. The Bacillus mesentericus group of bacteria was found to be most universally present on wheat and flour.

Greaney and Machacek, 1941, (8) carried out an extensive survey on the prevalence of seed-borne fungi on cereals from certain seed inspection districts in Canada. These investigators found species of Alternaria to be the most common fungi on wheat, oats, barley and rye; and species of Helminthosporium and Fusarium to be the most important disease-producing organisms.

Leach, 1944, (15), carrying out laboratory experiments under controlled conditions, found various fungi developing on heating wheat. They consisted chiefly of species of Penicillium, Aspergillus and Fusarium, with Cladosporium herbarum, types of Alternaria tenuis, various yeasts and species of Monilia occurring less frequently.

Gordon, 1944, (7) dealing specifically with the genus Fusarium, found that in the 1448 farm samples of seed of common wheat tested species of this fungus were present in approximately 39% of the samples; and of the 262 samples of durum wheat 38% showed species of Fusarium to be present. However, species of this fungus were isolated only from a small percentage of the seed (.66% of common wheat and .50% of durum wheat).

Investigation of other grains and various seeds has also been undertaken. Thom and Le Fevre, 1921, (25) studied the flora of cornmeal. They felt that it might be possible, by routine culturing, to determine the species represented and something of their relative abundance in the sample. The following bacteria were isolated: micrococcci, mesentericus, the colon-aerogenes group and lactobacilli with the morphological and cultural characteristics of organisms of the pickle and sauerkraut form. Molds such as Aspergillus repens, A. niger, A. flavus, Fusarium sp., various Mucor spp. and the occasional green species of Penicillium were found. A freshly milled sample of meal contained 1,000,000 bacteria per gram (60% of which were acid producers) and 100,000 molds per gram of meal.

Bakke and Noecker, 1933, (2) isolated Aspergillus niger, A. flavus, Rhizopus sp., Fusarium sp., Penicillium sp. and various species of bacteria from oats. Irregularities in oxygen consumption and temperature of oats packed in flasks were thought to be due to variation in the fungal flora of the different seed lots.

Ramstad and Geddes, 1942, (19) isolated a wide variety of fungi from soybeans: Alternaria sp., Fusarium sp., Penicillium sp., the

Aspergillus repens group, the A. niger group, Chaetotheca sp., Verticillium sp., the Aspergillus flavus-oryzae group, Mucorales, Rhizopus nigricans and Acrostalagmus sp.

A detailed investigation of the microflora of soybeans was undertaken by Ian W. Tervet (23). Soybeans were surface sterilized and then plated on potato dextrose agar. Species of Alternaria developed the most frequently, with other species in the following order: Fusarium sp., Aspergillus sp., Rhizopus nigricans, Chaetomium sp., Cephalothecium roseum and Trichoderma viride. Seeds stored at 45°C. showed a maximum degree of infection.

NUMBERS OF ORGANISMS IN WASHINGS FROM WHEAT

Two sets of samples were provided through the courtesy of the chemist-in-charge, Board of Grain Commissioners for Canada, Winnipeg. The first set was taken from individual box-cars shipped from various points in Western Canada during the period May 1, 1943 to January 15, 1944. It included 12 samples of No. 1, 12 of No. 2, 6 of No. 3 and 6 of No. 4 Manitoba Northern grades of red spring wheat. The second set will be referred to later.

In order to get reliable estimates of the populations, tests were run on six replicate portions of each sample. Each portion consisted of 10 grams, which was transferred aseptically to a 6 oz. glass screwtop bottle containing 90 ml. of sterile water and a small quantity of sterile gravel. The bottles were then shaken for 30 minutes by a mechanical shaking device. All plating was carried out in a room which was previously

sprayed with a disinfecting solution of chlorine.

Preliminary tests were made on each sample to determine the dilution which could be counted with the most accuracy. Two plates were prepared from the dilution, selected as above for bacteria, two from the dilution for yeasts and two from the dilution for fungi. This gave an estimate of bacteria on one sample representing the average from 12 plates and an estimate of yeasts and of fungi based on the same number of plates.

Nutrient agar medium was used as a substrate for bacterial growth and Czapek's agar, plus 0.5 ml. of 10% lactic acid per 100 ml. of medium, for yeasts and fungi. The addition of this amount of lactic acid appreciably inhibited bacterial growth, thereby encouraging a more rapid and luxuriant development of fungi.

In preliminary trials two temperatures of incubation were used, 25°C. and 37.5°C., the latter temperature being employed in the hope that organisms with high optimum growth temperatures might be isolated. However, since counts at 37.5°C. were much lower than those at 25°C., and differed widely in replicate plates from one sample, study at this termperature was discontinued.

The fungi and yeasts were counted throughout from the 1:100 and 1:1000 dilutions, respectively. Differences in bacterial counts were more varied and necessitated the use of different dilutions, depending on the grade of wheat under consideration. Estimates on grade 1 samples usually were based on plates from the 1:10,000 dilution; on grades 2 and 3 from either 1:10,000 or 1:100,000 dilutions; on grade 4 from the 1:100,000 dilution; and on grades 5 and 6 from dilutions as high as 1:1,000,000. The results are presented in tables 1a, 1b and 1c.

Table 1a. Bacteria washed from box-car samples of Manitoba Northern red spring wheat, Grades 1, 2, 3 and 4.

(100 thousands per gm. expressed as logarithms, averages from 12 plates)

Replications	Grades			
	1	2	3	4
1	0.78	0.89	1.06	2.29
2	0.98	1.24	1.00	1.85
3	0.30	0.84	0.98	1.79
4	0.00	0.89	1.56	1.37
5	0.36	1.32	1.60	1.88
6	0.71	0.00	1.76	1.90
7	0.76	0.79		
8	0.42	1.51		
9	0.59	0.85		
10	1.00	0.97		
11	0.32	1.42		
12	0.00	1.50		
$\bar{x}$	0.52	1.02	1.33	1.85
$s_{\bar{x}}$	0.10	0.12	0.14	0.12
t	5.20	8.50	9.50	15.42
d.f.	11		5	
5% level for t	2.20		2.57	

Table 1b. Fungi washed from box-car samples of Manitoba Northern red spring wheat, Grades 1, 2, 3 and 4.

(Hundreds per gm. expressed as logarithms, averages from 12 plates)

Replications	Grades			
	1	2	3	4
1	0.83	1.06	1.14	1.29
2	0.90	0.83	1.08	0.74
3	0.70	1.01	0.83	0.62
4	0.46	0.91	1.21	1.28
5	1.73	0.91	1.32	0.99
6	0.86	1.42	0.61	1.09
7	1.06	0.81		
8	1.47	1.33		
9	0.87	1.13		
10	0.88	0.72		
11	0.85	1.06		
12	0.72	1.16		
$\bar{x}$	0.94	1.03	1.03	1.00
$s_{\bar{x}}$	0.10	0.06	0.11	0.11
t	9.40	17.16	9.39	9.09
d.f.	11		5	
5% level for t	2.20		2.57	

Table 1c. Yeasts washed from box-car samples of Manitoba Northern red spring wheat, Grades 1, 2, 3 and 4.

(Thousands per gm. expressed as logarithms, averages from 12 plates)

Replications	Grades			
	1	2	3	4
1	1.59	0.84	0.65	1.30
2	1.66	1.09	0.77	1.86
3	1.06	1.39	1.26	1.52
4	0.78	0.75	0.43	1.52
5	1.21	0.75	1.81	1.61
6	1.56	0.00	0.82	1.02
7	1.05	1.34		
8	0.96	1.59		
9	1.47	1.06		
10	1.39	1.60		
11	0.02	1.46		
12	0.24	1.64		
$\bar{x}$	1.08	1.13	0.96	1.47
$s_{\bar{x}}$	0.15	0.14	0.20	0.12
t	7.20	8.07	4.80	12.25
d.f.	11		5	
5% level for t	2.20		2.57	