THE MICROFLORA OF CEREALS

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INTRODUCTION

The production of cereal crops is an important factor in the economy of Canada. Among the leading grain-producing countries of the world, Canada ranks fourth in wheat and oats and fifth in barley and flax, both with respect to acreage and output (1). The three prairie-provinces, Manitoba, Saskatchewan and Alberta, are Canada's granary as shown in Table 1.

Table 1

Cereal Production in Canada

Crop	Canada (bushels) 1925[1]	Prairie Prov. (per cent) 1925[1]	Canada (bushels) 1944[2]	Prairie Prov. (per cent) 1944[2]
WHEAT	411 375 700	78.34	416 635 000	94.02
OATS	513 384 000	62.77	499 643 000	74.21
BARLEY	112 668 300	83.56	194 712 000	73.96
FLAX	9 297 100	98.29	9 668 000	97.28

1) Canada Year Book 1926
2) Canada Year Book 1946

Nesbitt (27) calculated the value of grain production in the three Prairie Provinces over a period of 32 years, 1910 to 1941 inclusive. His calculation was based on the net price at the farmer's local shipping point, not including freight and handling charges:

Wheat	$	7 982 234 000
Oats	$	2 492 835 000
Barley	$	818 668 000
Flax	$	279 839 000

Including rye and mixed grains the total value amounted to $ 11 755 308 000 of which nearly eight billion dollars came from wheat alone. Canada supplies about 40 per cent of the world's export wheat and is thus the world's largest exporter.

Oats ranks second in total value, the greatest volume being produced in the Prairie Provinces. During the ten year period from 1925 until 1934 the total export of oats from Canada fluctuated between 2 and 34 million bushels per annum. About 15 million bushels are used annually by the milling industry, either in the production of food for human consumption or for milled feeds (10).

Barley holds the third place in acreage, total yield and value. The acreage has fluctuated widely since 1910, doubling during the first and second world wars. The annual acreage in Canada is approximately three and three-quarter million acres, about three million acres being grown in the Prairie Provinces (7). This was true until 1940. By 1944 the acreage had more than doubled to reach 7.3 million acres in Western Canada alone. Between 10 and 40 million bushels are exported yearly.

Flax in Canada is produced mainly for its seed and not for its fibre. Ninety to 97 per cent of the total crop is seed flax. The acreage declined from 2 million acres in 1912 to 243 600 acres in 1933. In 1944 the area under flax

was again greater than two million acres. The export of flax is negligible, but oilcake and oilcake meal are being exported in large quantities to Great Britain (25).

In Canada cereal crops have received a great deal of attention from plant-breeders, cereal chemists and plant-pathologists, but almost none from bacteriologists. It would appear desirable, both from a scientific and practical point of view to obtain fundamental information on the normal microflora of cereal seeds.

Several important and acute problems exist which can be traced to cereal seeds, though their exact role is not well understood. The feeding of cereal seeds to animals sometimes produces diseases. Blue-comb disease in chickens is attributed to wheat seeds. However, it is not known whether the disease is nutritional or microbial in origin. Spoilage of grain in storage is one of the most important economic problems in grain-producing countries. While several theories have been proposed, the real cause for the heating of grain is still a matter of conjecture. Milled products, particularly wheat flour, often possess undesirable qualities which make them unfit for consumption. Here again the true factors causing the trouble are not known. It is of interest to note in this connection that only very scanty attention has been paid to the microflora of the seeds involved.

The current search for antibiotics also brings the microbial population of seeds into greater prominence. Although the actual role of the normal microflora on plant-parts is unknown at the present time, attempts are being made to clarify the relationship between saprophytic organisms and plant-pathogens. This relationship appears to lend itself particularly well to the study of antibiotic phenomena.

Before any of these problems can be approached with assurance, it appears necessary to have information on the numbers and types of microorganisms on cereal seeds. The mechanism by which epiphytic bacteria reach the plant and the seeds must be fully understood before the occurrence of certain bacteria on seeds and plants can be satisfactorily explained.

This investigation was an attempt to obtain information on the bacteriological aspects of these problems. The microflora of commercial samples of Canadian flax, barley and oat seeds and of growing wheat plants was studied. This study was divided into three parts:

I. Numbers and proportions of microorganisms on seeds.

II. Taxonomy of the two main species of bacteria which were regularly encountered.

III. Microflora of growing wheat plants and the mechanism by which the bacteria may be transmitted from seed to plant and from plant to seed.

Part I

NUMBERS OF ORGANISMS ON SEEDS

Numbers of Organisms on Commercial Samples

Material

The seed samples tested were supplied through the courtesy of the Chemist-in-Charge, Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, Manitoba. Each sample was a 150 gram composite of certain statutory grades of flax, barley and oat seeds. A detailed description of each grade may be found in the **Definitions** of Statutory Grades of Western Grain[1)].

The samples were collected at monthly intervals for four months in 1946 and four months in 1947. Unfortunately, some of the best and some of the lowest grades were not available at every sampling.

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1) Definitions of Statutory Grades of Western Grain, Dep. of Trade and Commerce, Ottowa, 1939, 32 pp.

Other references dealing with the Canadian grain grading system and explaining it in some detail are:

Amendments to the Canada Grain Act, 1930. Canadian Wheat Pool, June 1930, 14pp.

Malloch, J. G., Canadian Wheat. Dep. of Trade and Commerce, Ottowa, 29 pp.

Anderson, J. A., Canadian Barley. Jour. of Inst. of Brewing 33 (N.S.), Jan. 1936, p. 35-44.

Lehberg, F. H., and Anderson, J.A., Western Canadian Flaxseed. Sci. Agr., 21, Aug. 1941, p. 727-745.

Flax. Flax is graded Western Canada No. 1, 2, 3 and 4. Grade C.W. 4 passes only rarely through trade channels and, consequently, was tested only twice each year.

Barley. Commercial grades of barley seeds are grouped as:

C.W.	Two-Row	No. 1 and 2
C.W.	Six-Row	No. 1, 2 and 3
C.W.	Yellow	No. 2 and 3
Feed Grades		No. 1, 2 and 3

Yellow stands for Canada Western Six-Row Smooth-Awned Yellow Barley, which has smooth awns differing thus from other six-row varieties. C.W. Six-Row No. 1 and C. W. Two-Row No. 1 pass only rarely through Winnipeg. C.W. Two-Row No. 1 was obtained only once in 1946 and not at all in 1947, while C.W. Six-Row No. 1 was not obtainable in either year. C.W. Yellow No. 2 was tested twice in 1947, but not in 1946.

Oats. Commercial grades of oats are grouped as:

C.W. No. 1, 2, extra 3 and 3

Feed Grades extra 1, No. 1, 2 and 3

C.W. No. 1 was tested only twice in 1946 and not in 1947. Extra No. 1 Feed was obtained three times each year. All other grades were tested four times yearly.

A two-year old sample of hulless oats, variety Brighton, was obtained through the courtesy of the Senior Cerealist of the Dominion Laboratory of Plant Breeding, Winnipeg.

The sample was tested in 1946 and again in 1947, thus furnishing a comparison between hulless and hulled seed. The results were used to show the effect of storage on numbers of micro-organisms on seeds.

Methods

Methods for determining the number of microorganisms on seed samples were those used by James et al. (21) when working with wheat. The results obtained with flax, barley and oats are, therefore, directly comparable to those reported for wheat. Three 10 gram portions of each grade were used each time, and the results were expressed as the average number on duplicate plates. The data were analyzed by appropriate statistical methods.

Only those counts were included in the analysis of variance which were available an equal number of times in both years. This implies that the results of the analysis refer to samples most commonly encountered in commerce. The complete set of data and tables necessary for statistical calculations are presented in the appendix.

Results

Flax

The number of microorganisms washed off the smooth seed-coat of flax seeds was exceedingly large. Millions of bacteria, hundreds of thousands of yeasts and thousands of fungi were obtained from one gram of seed.

The average numbers of bacteria, yeasts and fungi were calculated per gram of seed from the results of 12 replications, unless stated otherwise. These are presented in Table 2.

Table 2

Average Numbers of Organisms on Flax Seed -- per Gram

Grade	No. Repl.	Bacteria x 10^6 1946	1947	Yeasts x 10^4 1946	1947	Fungi x 10^3 1946	1947
C.W.1	12	13.4	24.5	29.7	14.6	1.3	1.7
C.W.2	12	91.9	55.2	31.2	45.5	2.1	2.9
C.W.3	12	76.6	35.6	17.7	48.2	4.2	7.1
C.W.4	6	6.1	39.2	6.4	10.8	5.9	51.7

The numbers of fungi increased regularly from grade to grade, while those of bacteria and yeasts did not show a similar trend. C.W. No. 2, the grade most commonly found in trade, harboured the largest number of bacteria in both years.

The analysis of variance for bacteria, yeasts and fungi for both years is listed in Table 3. C.W. No. 4 was tested only twice each year and was not included in the analysis.

Table 3

Analysis of Variance for Bacteria, Fungi and Yeasts

Source	D.F.	Mean Square 1946	F	Mean Square 1947	F	5%
		BACTERIA				
Months	3	2 176 999.79	150.30	1 104 749.66	17.50	3.01
Grades	2	2 076 241.00	143.35	290 733.25	4.61	3.40
M x G	6	1 394 211.67	96.26	67 385.10	1.07	2.51
Error	24	14 483.97	-	63 110.86	-	-
		FUNGI				
Months	3	148.78	0.76	3 054.13	13.73	3.01
Grades	2	2 835.08	14.43	9 882.11	44.43	3.40
M x G	6	149.53	0.76	2 703.30	12.15	2.51
Error	24	196.47	-	222.42	-	-
		YEASTS				
Months	3	27 520.41	1.90	1 008 793.11	4.62	3.01
Grades	2	65 815.36	4.55	418 884.34	1.92	3.40
M x G	6	70 073.10	4.83	555 426.22	2.54	2.51
Error	24	14 449.47	-	218 532.03	-	-

It is apparent from the above analysis that there is little consistency in counts of any of the groups of organisms considered. A summarized statement on the statistical study of the counts of micro-organisms on the three grains - flax, barley and oats - appears on page 15.

Barley

Counts obtained from barley were similar to those obtained from flax. The number of bacteria washed off the seed was strikingly large, while the number of fungi was small in comparison. Yeasts occupied an intermediate position.

Table 4 lists the average numbers of bacteria, yeasts and fungi, calculated per gram of seed. Since C.W. Two-Row No. 2 was only available three times in 1946, the results of this year constitute the average of 9 replications, while in 1947 they constitute the average of 12 replications.

Table 4

Average Number of Organisms on Barley Seed -- Per Gram

Grade	No. Repl.	Bacteria x 10^6 1946	1947	Yeasts x 10^4 1946	1947	Fungi x 10^3 1946	1947
Two-Row							
C.W. 1	3	0.1	-	0.8	-	4.4	-
C.W. 2	9 & 12	2.3	11.6	10 .0	8.6	1.8	2.2
Six-Row							
C.W. 2	12	20.8	16.2	16.1	11.4	6.0	8.4
C.W. 3	12	32.7	40.0	29.3	12.0	11.9	10.5
Yellow							
C.W.2	6	-	0.7	-	25.5	-	2.4
C.W.3	12	12.9	8.9	18.2	31.9	16.2	2.4
Feed							
No. 1	12	14.5	12.4	35.8	20.7	10.7	4.2
No. 2	12	25.3	24.0	22.1	32.4	9.6	23.6
No. 3	12	24.2	49.6	23.1	34.8	21.8	11.7

Two-row barleys harboured fewer organisms than the six-row grades. The number of rows appears to have little effect on counts when the two-row results are compared with those of the yellow six-row grades.

Feed grades do not seem to harbour more organisms than ordinary six-row grades.

The analysis of variance for bacteria, yeasts and fungi for both years is shown in Table 5. The grades C.W. Two-Row No. 1 and 2, and C.W. Yellow No. 2 were not included because they were not always obtainable.

Table 5

Analysis of Variance for Bacteria, Fungi and Yeasts on Barley

Source	D.F.	Mean Square 1946	F	Mean Square 1947	F	5%
		BACTERIA				
Months	3	85 016.93	5.10	321 303.21	35.44	2.80
Grades	5	65 071.06	3.90	317 564.76	35.03	2.41
M x G	15	14 499.40	0.87	79 726.70	8.79	1.88
Error	48	16 668.14	-	9 065.92	-	-
		FUNGI				
Months	3	371.65	2.02	43 189.01	0.75	2.80
Grades	5	371.49	2.02	67 550.41	1.17	2.41
M x G	15	188.24	1.02	72 718.55	1.12	1.88
Error	48	183.86	-	57 632.87	-	-
		YEASTS				
Months	3	2 975.09	5.25	252 268.16	5.09	2.80
Grades	5	647.11	1.14	135 374.21	2.73	2.41
M xG	15	648.37	1.14	85 940.83	1.73	1.88
Error	48	566.29	-	49 535.21	-	-

Oats

The numbers of bacteria, yeasts and fungi washed off oat seeds showed the same pattern as those of the other two crops. Bacteria were considerably more numerous than yeasts and fungi. The difference between the feed grades and C.W. grades was much more pronounced than in the case of barley. Table 6 shows the average number of microorganisms per gram.

Table 6

Average Number of Organisms on Oats -- per Gram

Grade	No. Repl.	Bact. x 10^6 1946	1947	Yeasts x 10^4 1946	1947	Fungi x 10^3 1946	1947
C.W. 1	6	0.9	-	3.9	-	2.5	-
C.W. 2	12	5.9	3.0	17.8	4.6	10.6	5.0
C.W. extra 3	12	3.6	2.1	17.3	5.3	8.8	12.6
C.W. 3	12	5.4	5.6	16.5	8.3	16.2	4.2
Feed extra 1	9	7.2	7.6	18.2	6.7	14.4	7.3
Feed No. 1	12	5.4	6.1	22.1	10.9	13.6	8.1
No. 2	12	10.8	12.5	20.3	13.0	39.5	25.6
No. 3	12	10.5	16.4	26.7	17.2	33.0	39.2

While the difference in counts between C.W. No. 1 and the other grades was considerable; differences between C.W. No. 2 and C.W. Extra No. 3 were only slight. Feed grains had more organisms than the C.W. grades in both years.

The analysis of variance is presented in Table 7. The grades C.W. No. 1 and Feed Extra No. 1 were not included in the analysis.

Table 7

Analysis of Variance for Bacteria, Yeasts and Fungi on Oats

Source	D.F.	Mean Square 1946	F	Mean Square 1947	F	5%
		BACTERIA				
Months	3	29 924.11	26.17	37 404.18	13.14	2.80
Grades	5	10 677.57	9.34	38 302.79	13.45	2.41
Mx G	15	2 845.10	2.49	9 107.80	3.22	1.88
Error	48	1 143.61	-	2 846.67	-	-
		FUNGI				
Months	3	2 453.44	8.35	678 930.53	29.83	2.80
Grades	5	1 971.05	6.71	354 457.08	15.57	2.41
M x G	15	964.28	3.28	134 541.38	5.91	1.88
Error	48	293.93	-	22 757.78	-	-
		YEASTS				
Months	3	389 689.89	12.68	17 834.29	2.52	2.80
Grades	5	17 750.01	0.58	27 779.56	3.93	2.41
M x G	15	40 909.11	1.33	23 683.79	3.35	1.88
Error	48	30 737.61	-	7 061.65	-	-

Most of the variation in counts of bacteria on the three grains was due to time of sampling, rather than to grades. In all instances, except one, the mean squares for months were greater than those for grades. However, grades, as well as months of sampling, were important sources of variation.

Variations in counts of fungi on oats were due to grades as well as to months in both years of the test. In the case of barley neither grades nor months appeared to influence

the variation in counts. In the case of flax, grades were the main source of variation in both years. Time of sampling was an important factor in 1947, but not in 1946.

Variation in counts of yeasts was mostly due to month of sampling, as shown by the large mean squares for months in four out of six instances. In 1946 grades were an important source of variation in flax, but not in barley and oats, while in 1947 the reverse was true.

Since in many cases the interaction between grades and months of sampling was large, it is apparent that samples of one age should be used in an attempt to evaluate the effect of grades on counts.

Hulless Oats

A sample of hulless oats, variety Brighton, was obtained through the courtesy of the Senior Cerealist, Dominion Laboratory of Plant - Breeding, Winnipeg. The history of this sample was known. The variety was grown at the Dominion Experimental Farm Brandon, Manitoba. The seed was sent to Winnipeg where it was stored. It was tested in 1946, and again, in 1947, after two and three years of storage.

This sample furnished a means for showing the effect of hull and other probable factors on counts. Table 8 shows the difference between hulled and hulless oats. The figures for the hulled grades were taken from Table 6. The age of these samples was not known, while that of the hulless variety was two years.

Table 8

Comparison between Hulled and Hulless Oats

Grade	No. Repl.	Bacteria x 10^5	Yeasts x 10^4	Fungi x 10^3
C.W. 1	6	9.4	3.9	2.5
C.W. 2	12	59.3	17.8	10.6
Hulless	12	2.3	1.3	5.3

The average number of bacteria, fungi and yeasts per gram on the hulless sample after two and three years of storage is listed in Table 9. Also shown in the table is the reduction in numbers which had taken place.

Table 9

Average Number of Organisms on Hulless Oats and Percentage Reduction of Counts from the Second to the Third Year of Storage

Year	No. Repl.	Bacteria x 10^5	Yeasts x 10^4	Fungi x 10^3
1946	12	2.3	1.3	5.3
1947	12	0.5	0.6	4.7
Percentage Reduction		80.14	55.34	11.09

Surface Colonies on Nutrient Agar Plates

Among the surface colonies on nutrient agar plates were two types which occurred with striking regularity and in great numbers. One type consisted of yellow-pigmented, circular and smooth colonies; the other of whitish-transparent colonies which fluoresced when cultured in appropriate media. In order to estimate the frequency of occurrence of these two types, the following procedure was chosen. First, the number of surface colonies in proportion to the total count on each grade was determined. Then, the number of both types in proportion to the total count of surface colonies was calculated.

Table 10 shows the proportion of surface colonies to the total count on a percentage basis. The figures from which these ratios were calculated are listed in the appendix (Table 69). In most instances they were obtained from 12 replications. The actual number of replications for each grade may be found in Tables 2, 4 and 6.

Table 10

Ratio of Surface Colonies to the Total Count

(in per cent of total count)

Grade	FLAX 1946	1947	BARLEY		1947	OATS 1946	1947
Dilution	10^5	10^4			10^5	C.W.Grades 10^4 Feed Grades 10^5	
C.W. 1	16.30	19.96	C.W.	2	8.70	C.W. 1 10.68	-
C.W. 2	16.83	13.93	Two Row			C.W. 2 19.36	14.61
C.W. 3	15.80	19.25	C.W.	2	9.21	C.W. ex.3 17.36	15.31
C.W. 4	20.02	16.58	Six Row	3	6.74	C.W. 3 14.52	10.43
			C.W.	2	9.49	Feed ex.1 -	12.92
			Yellow	3	11.91	Feed 1 15.59	12.41
						2 14.39	11.07
			Feed	1	9.49	3 14.63	8.61
			Grades	2	10.08		
				3	5.01		
All Grades	16.43	16.79			9.43	15.60	12.02

It is apparent from the above table that the percentage of surface colonies in relation to the total count was fairly constant. It was constant from year to year and, also, from grade to grade within each cereal. This constancy was made use of in determining the occurrence of these two types on cereal seeds.

Yellow colonies, identical in appearance, were counted and recorded. The whitish colonies were tested for fluorescence in appropriate media before being considered as Type II. The numbers of Type I and Type II were added together and expressed as a percentage of the total number of surface colonies. This information is presented in Table 11.

Table 11

Percentage of the Two Main Types among Surface Colonies

Data for 1947

FLAX		BARLEY		OATS	
Grade	Percentage	Grade	Percentage	Grade	Percentage
C.W. 1	83.10	C. W. 2	71.47	C.W. 2	76.10
C.W. 2	90.56	Two-Row		C.W. ex.3	80.36
C.W. 3	83.82	C.W. 2	79.94	C.W. 3	73.55
C.W. 4	74.67	Six-Row		Feed ex.1	62.71
		3	68.00	Feed No.1	72.74
		C.W. 2	76.06	No.2	66.36
		Yellow		No.3	70.59
		3	73.54	Hulless	83.87
		Feed			
		Grade 1	80.49		
		2	74.87		
		3.	75.00		

Table 11 shows that both types accounted for approximately 80 per cent of all surface colonies. It does not show, however, the following two features:

1. The ratios of yellow to fluorescent colonies often varied considerably from grade to grade. Yet, the total of the two varied only little in relation to the number of surface colonies.
2. There were three to seven times as many yellow colonies as fluorescent ones.

From the evidence presented in Tables 10 and 11, there can be little doubt that these two types constitute the predominating bacterial flora on seeds. For this reason, a detailed taxonomic study of these two types was undertaken. This is reported in Part II.

Bacterial Spores and Coliform Bacteria on Seeds

While determining the number of microorganisms on commercial samples by the plate-count method, two of the dilutions of the wash-water, prepared for plating purposes, were set aside for special investigations. One was undertaken to determine the number of bacterial spores on seeds; the other to determine the presence of coliform bacteria.

Numbers of Bacterial Spores

The 1:100 dilution of the wash-water was heated for half an hour at approximately 90 degrees C.: the assumption being that only spores of bacteria would survive this treatment.

Then, one cubic centimeter of the heated wash-water was plated in duplicate on nutrient agar and incubated for 5 days at 26 degrees C.: which was the procedure used to determine the numbers of organisms on seeds. The experiment was repeated three times for each sample. Detailed counts are listed in the appendix (Tables 62-68). The calculated average number of spores per gram of seed is shown in Table 12. This experiment was carried out in 1947.

Table 12

Average Number of Bacterial Spores per Gram

FLAX		BARLEY			OATS		
Grade	Nos.	Grade		Nos.	Grade		Nos.
C.W. 1	780	C.W. Two-Row	2	420	C.W. 2		310
C.W. 2	980				C.W. ex.3		440
C.W. 3	990	C.W. Six-Row	2	370	C.W. 3		510
C.W. 4	1770		3	470			
		C.W. Yellow	2	620	Feed extra 1		530
			3	1410	Feed No.1	15	440
						2 16	950
		Feed Grade	1	1310		3 20	000 over
			2	900	Hulless		363
			3	1110			

Although the lower grades of flax and barley had more spores than the better grades, the differences between grades were not significant.

In the case of oats, the difference between the C.W. grades and feed grades was conspicuous. Feed grades harboured approximately 40 times as many bacterial spores as the other grades.

Coliform Bacteria

> The coliform group is a large one made up of closely related, highly intergrading and somewhat unstable bacteria, which form a continuum from the lactose-negative paracolon forms to highly reactive Aerobacter aerogenes (28).

The accurate determination of the presence of coliform organisms on cereal seeds is a major task from a bacteriological point of view. Their presence may be of sanitary importance. Tanner (35) mentioned coliform bacteria in dough used for bread-making, and in water used to wash whole wheat, and, consequently, in the flour milled from this wheat. Rogers, Clark and Evans (30), 1915, isolated coliform bacteria from grains and stated that Klein and Houston, 1900, Papasotiren, 1902, and Metcalf, 1905, also found coliform bacteria on grain, but that Winslow and Walker, 1907, could not find any on 178 samples of grain.

In view of this conflicting evidence it was decided to look for presumptive evidence of the presence of coliform bacteria on wheat, flax, barley and oat seeds.

During the preliminary experiments, the wash-water from 10 gram samples of grain was obtained in the usual manner and one cubic centimeter of each dilution was plated

on violet red bile agar according to instructions [1]. Typical colonies developed on all plates from all samples after 3 days at 37 degrees C. However, when these colonies were picked and transferred to brilliant green lactose bile broth (Difco) no turbidity developed at 37 degrees C.

This experiment was repeated and enlarged. Using four samples of seeds, consisting of the best grades of wheat, flax, barley and oats, the same results were obtained. This time, the apparently negative brilliant green lactose bile broth cultures were streaked on Levine's eosin methylene blue agar and incubated at 37 degrees C. Colonies developed after 24 hours which looked like Escherichia coli and Aerobacter aerogenes, according to the description given in the Difco Manual (1943, p.27). However, when these colonies were picked and transferred to standard lactose peptone broth only slight turbidity developed and gas was not formed. E. coli cultures, carried along as checks, gave positive results in all cases.

It appeared from these preliminary experiments that the procedure used failed to give presumptive evidence of the presence of coliform bacteria, and that the "typical" colonies on the solid media gave false positive results.

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1) Standard Methods for the Examination of Dairy Products. 8 th ed., American Public Health Assoc., New York, 1941, 288 pp.

Since it was necessary to use higher dilutions in order to obtain plates with only a few colonies, the possiblity that the coliform bacteria might have been diluted out must be taken into consideration. Consequently, the problem was approached from a different direction.

Ritter (29), 1919, Horwood and Heifetz (16), 1934, Shunk (33), 1934, and Farrell (14), 1935, have shown that brilliant green lactose bile broth and standard lactose broth are the best combination of media to show presumptively the presence of coliform bacteria.

The 1:10 dilution of the wash-water was used this time. One cubic centimeter was added to 10 ml of standard lactose broth. The tubes were incubated for 24 hours at 37° C. and checked for gas. The presence of gas in both media was taken as evidence that coliform bacteria were on the grain. Results are listed in Table 13.

The results were highly irregular. Aliquot portions of a sample sometimes contained coliform bacteria; sometimes they did not. This may be taken as an indication that the number of coliform organisms was very small. Of 100 tests only 18 were positive for both media.

Table 13

Presumptive Evidence of Coliform Bacteria on Seeds

FLAX			BARLEY			OATS		
Grade	I	II	Grade	I	II	Grade	I	II
C.W.1	+	+	C.W. Two-Row 2	-	-	C.W.2	-	-
	-	-		-	-		-	-
	-	-		-	+		-	+
	+	+		-	+		-	-
	+	+		-	+		-	+
	-	-					-	+
C.W.2	-	-	C.W. Six-Row 2	+	-	C.W.	-	-
	-	-		-	+	extra 3	-	+
	-	-		+	+		-	-
	-	-		+	+		-	-
	-	-		-	+		-	-
	-	+					-	+
C.W.3	-	-	C.W. Six-Row 3	-	+	C.W.3	-	+
	-	+		-	+		-	+
	-	-		-	+		-	-
	+	-		-	+		-	-
	+	+		+	+		+	+
	+	+					-	-
			C.W. Yellow 2	-	-	Feed	-	+
				-	-	extra 1	-	+
							-	-
			C.W. Yellow 3	-	-	Feed 1	-	+
				+	+		-	-
				+	+		-	-
				-	+		-	-
				-	+		-	+
							-	+
			Feed No. 1	+	-	Feed 2	+	-
				-	+		-	+
				+	+		-	-
				-	+		-	-
				-	+		+	+
							+	+
			Feed No. 2	+	+	Feed 3	-	+
				-	+		-	+
				-	+		-	-
				+	-		-	-
				-	+		-	+
							+	+
			Feed No. 3	-	-	Hulless	-	-
				-	+		-	+
				-	+		-	+
				+	+		-	-
				+	+		-	-
							-	+
Number of Tests			18		37			45
Positive tests			5		9			4

B.G.L.B.- I
St.L.Br.- II

Discussion

Comparisons of findings of various investigators who studied the quantitative relationships of microorganisms on cereal seeds are difficult to make because:

1. different investigators used different techniques
2. counts were expressed per gram of seed or per kernel
3. one gram of seed contains a variable number of kernels
4. the history of the material under test was not known
5. in one instance (38) results were given without the methods by which they were obtained.

The only comparison that can be made is the one between the findings of James et al. (21) with wheat and the results with flax, barley and oats reported in this study.

This investigation and the results of other investigators indicate clearly that there exists an enormous bacterial population on seeds. Some of these findings are listed in Table 14.

Table 14

Counts of Bacteria by other Investigators (x 1000)

	Per Kernel				Per Gram
	Duggeli	Woller	Mack	Hoffmann[1)]	Duggeli
BARLEY	3 - 80	12 - 24	50	7 740	1 600
OATS	1.7- 35	4.6- 23	200	11 640	1 330

1) quoted by Duggeli

If an attempt is to be made to explain the presence of enormous numbers of bacteria on seeds the following factors must be taken into consideration:

1. epiphytic bacteria which proliferate on the seed
2. certain bacteria which are accidentally there
3. physical conditions of the seed coat
4. size of the kernel
5. physiological changes within the seed
6. seasonal influences
7. storage and handling
8. interaction of some of these factors

Epiphytic Bacteria. It was found in this investigation that approximately 80 per cent of the bacteria belonged to two species. The regular occurrence on all samples tested and the large proportion of these two types seem to indicate that conditions on the seed are favourable for the existence and active multiplication of these bacteria. Burri (9), 1903, recognized the existence of epiphytic bacteria and explained their large numbers by suggesting that they proliferate vigourously on seeds and plants. This view was confirmed by several investigators (12, 15, 24, 26, 34).

Accidental Contaminants. The other 20 per cent of bacteria observed on seeds consisted of cocci, gram negative rod-shaped bacteria, which occurred only infrequently on the plates, sporeforming and coliform bacteria. The small

number and the irregularity of their presence on seeds seems to indicate that the conditions on the seed do not favour their multiplication to any large extent. These forms may be considered to be accidental contaminants on grain.

Physical Conditions of the Seed. The morphological structure of the seed coat may influence the number of bacteria. The comparison of hulless and hulled oats, reported in this work, revealed that hulless kernels harboured considerably fewer bacteria than hulled. However, the different ages of the samples which were compared may have had something to do with the difference in numbers. The exact role of the hull awaits further clarification.

The soundness of the seed coat appears to be important. the results of this study indicate clearly that quality of the grain influences the counts of bacteria to a marked extent. Quality of grain is judged in Canada by "soundness" and "cleanness" of the seeds. "Soundness" refers to such characteristics as health, maturity, weathering, frosting, heat-damage, discoloration and sprouting "Cleanness" refers to the freedom of admixtures, such as weed seeds. Samples of lower quality generally contained larger numbers of bacteria. James et al. (21), 1946, showed that in the case of wheat there was a definite relationship between quality and numbers of bacteria: the poorer the

quality the larger was the count. These investigators also showed that weathered, immature and frosted wheat kernels harboured more bacteria than normal, healthy seed.

Size of Kernel. This seems to be a major factor in determining the number of bacteria in a given volume of seed. The number of bacteria on flax seed, which has a smooth seed-coat, was considerably greater than that on hulled seeds. This may be explained by the fact that flax seeds are much smaller than barley or oat kernels. Consequently, there would be a greater number of kernels and a larger surface area in a gram of seed.

Physiological Changes within the Seed. That physiological changes within the seed influence decidedly the number of bacteria on the seed was shown by Mack (24) in a comparison of unsprouted and sprouted seeds (average of 20 kernels):

	unsprouted	sprouted
Barley	0.5×10^5	50×10^5
Oats	2×10^5	380×10^5

Seasonal Influences, Storage and Handling. On the sample of hulless oats tested the number of bacteria decreased drastically from the second to the third year of storage. This sample was kept in a cup-board and was, therefore, not exposed to climatic influences. There can be little doubt that temperature and humidity affect grain during farm storage.

One might anticipate that under favourable conditions active multiplication would take place. In this study significant differences between months of sampling were obtained.

Associative Relationships. To what extent certain species of one class of microorganisms or species of different classes favour or suppress each other on the seed is a matter for further research. Hummer (19) mentioned that on barley seeds yeasts tend to eliminate bacteria in the struggle for survival. Zikes, quoted by Hummer, maintained that the predominating species of bacteria on seeds had a tendency to favour each other. Some investigators (26, 34) believed that a large bacterial population protected the seed and the plant by suppressing fungi, particularly pathogenic species.

The role of yeasts on seeds is somewhat obscure. Their numbers fluctuate widely. The large total count does not exclude the possibility that they multiply on the seed. It is doubtful, however, whether they can be considered to be epiphytes. For example, no yeasts were found on seedlings and young plants in the field. This may be taken as an indication that yeasts reach the plant from external sources, rather than from the seed. Their epiphytic nature would be established if it could be shown experimentally that they exist on the coleoptile and growing point of very young seedlings.

Fungi occurred in relatively small numbers. The large variety of types observed seems to indicate that fungi are not epiphytic on seeds. The soundness of the seed-coat appears to be an important factor, because it was found in this study that, in some instances, the number of fungi was larger on grades of poorer quality. James et al. (21), 1946, found that most of the fungi on wheat also occurred in soils.

Part II

THE TWO MAIN TYPES OF EPIPHYTIC BACTERIA

Description of Type I

Yellow pigmented colonies were regularly encountered on the surface of nutrient agar plates used to estimate the number of bacteria on cereals. Golden-yellow colonies which were of uniform appearance were considered to be Type I. Ten colonies were selected from nutrient agar plates prepared from each of the following seeds: wheat, oats, barley and flax.

On preliminary study two of the forty cultures were found to differ from the others in certain respects. The study of Type I, accordingly, was limited to 38 cultures. The remaining two cultures will be considered in a separate sub-section.

Morphology

Shape, arrangement and size were determined by making smears from 24 hour old nutrient agar plate cultures and staining them with 0.5 per cent safranin. The cells appeared as rods with rounded ends, averaging 1.6 by 0.8 microns, and occurring singly. On negative nigrosin mounts they were somewhat larger. The average length was 2.5 microns and the average width 1.0 microns.

Hucker's modification of Gram's stain showed these organisms to be Gram negative. Gray's flagella stain was used with good success. Most cells had one polar flagellum,

though some had two at one pole. Dorner's spore stain and Anthony's capsule stain prepared from agar plate cultures incubated for different periods failed to show spores or capsules.

Cultural Characteristics

Cultural characteristics were studied according to standard procedures, using Difco products. Incubation was for 5 days at 26 degrees C., except with gelatin, in which case it was for 4 weeks at 22 degrees C.

Nutrient Agar- colonies golden-yellow, circular, entire, with tiny indentations, slightly convex, glistening, smooth, having, on the basis of 115 measurements, an average diameter of 3.97 mm.

Since the diameters of colonies ranged from pin-point to 8 mm, it appeared desirable to determine whether this was due to normal variation or to hereditary transmission. On plating small and large colonies, it was found that each gave rise to both types of colony.

Further evidence that both types were produced by the same kind of bacteria was indicated by acid-production in 0.5 per cent sucrose nutrient broth, incubated for one month at 26 degrees C. Bacteria from each type of colony lowered the pH from 7.1 to 5.9.

It would appear, therefore, that the difference in the

size of the colony, in this case, was not due to characteristics inherent in the species.

Nutrient Agar - growth on streaks abundant, yellow, filiform, entire, slightly convex, glistening, smooth, not discoloring the medium, viscid and slimy on ageing.

Wort Agar (pH 5.0) - colonies oval to circular, markedly convex, glistening, smooth, deeply yellow, average diameter 3.5 mm. Colonies developed better on this slightly acid medium than on standard nutrient agar.

Growth on Glucose (1%) and Maltose (1%) Agar was exceedingly abundant. Spot-inoculations produced large colonies which showed the presence of zooglea under a magnification of 440 diameters. These appeared as denser areas within a colony. In no instance were they observed with the same ease and regularity as was stressed by other investigators.[1)]

Nutrient Broth - uniformly turbid, slightly better growth near the surface, sometimes forming a fine pellicle or leaving a ring on the test-tube; sediment viscid.

Gelatin - liquefaction slow, napiform; in two weeks infundibuliform, sediment yellow.

1) This phenomenon was a regular occurrence on solid media, according to Duggeli. He went so far as to claim that it was constant and hereditary for the species. Mack found zooglea on sugary media under low magnification, but none on nutrient agar. Huss did not find zooglea, but noticed the formation of calcium oxalate crystals.

Physiology

Fermentations. The isolates under study produced alkalinity in standard nutrient broth upon incubation at 26 degrees C. After one week the pH was raised from 7.2 to that of 7.8 to 8.5 in different cultures, and after two weeks to that of 9.0 to 10.3. It became obvious from this observation that beef-extract peptone broth was unsuitable for studying the acid-production of fermentable substances.

In a basal medium consisting of inorganic salts, any change in reaction may be ascribed to the fermentable substance added to it. For this reason the synthetic medium of Elrod and Braun was used: $MgSO_4$ 0.2%, CaCl 0.1%, NaCl 0.2%, K_2PO_4 0.2%, carbohydrate 1%. The media when made up differed in their initial pH due to varying degrees of dissociation of sugars during sterilization. They were inoculated from 24 hour old nutrient agar plate-cultures and incubated for six days at 26 degrees C. Determinations of the hydrogen-ion concentration were made with a Coleman Model 3 Electrometer. The results are presented in Table 15. Detailed results for each culture are listed in the appendix (Table 70).

Table 15

Change in pH produced by 38 cultures (15 sugars)

Carbohydrates		Check	Range	Aver. Diff.[2]
Monosaccharides				
Pentoses	Xylose	6.80	3.20 - 5.90	- 3.06
	Arabinose	7.00	3.45 - 6.00	- 2.72
	Rhamnose	7.95	4.70 - 7.75	- 1.37
Hexoses	Glucose	6.55	3.45 - 4.00	- 2.91
	Mannose	6.50	3.60 - 3.95	- 2.72
	Galactose	6.40	3.80 - 4.45	- 2.30
	Fructose	5.35	4.00 - 4.50	- 1.08
Disaccharides				
	Sucrose	7.10	4.70 - 6.90	- 1.12
	Maltose	6.40	5.25 - 6.30	- 0.37
	Lactose	6.80	6.00 - 6.70	- 0.32
Trisaccharides				
	Raffinose	6.80	5.55 - 7.15	- 0.04
Polysaccharides				
	Starch	7.40	7.00 - 9.00	- 0.33
	Dextrin	7.25	7.20 - 8.85	- 0.52
	Inulin	7.30	8.05 - 8.95	- 1.27
Glucosides				
	Salicin[1]	7.30	5.50 - 6.80	- 3.73

1) average of 7 readings only

2) calculated by taking the arith.aver. of the differences of 38 pH readings from the aver. of 5 uninoculated checks per sugar

It may be observed from these results that certain sugars were decomposed by the cultures to a greater extent than others. They were acidified in the following decreasing order:

Salicin, xylose, glucose, arabinose, mannose, galactose, rhamnose, sucrose and fructose.

Lactose and maltose were only slightly acidified.

Raffinose showed no change in reaction.

A few cultures produced a slightly acid reaction in polysaccharide media.

Nitrate Reduction. The ability to reduce nitrate was tested by culturing for three days at 26 degrees C. in standard nutrient broth to which 0.1 per cent potassium nitrate was added. The following reagents were used for the detection of nitrite:

Solution 1: 8 gm sulphanilic acid in 1 liter of 5 N acetic acid

Solution 2: 5 gm alpha-naphtylamine in 1 liter of 5 N acetic acid

All 38 cultures reduced nitrate to nitrite.

The addition of a few drops of Nessler's reagent to a replicate of each culture in the above nitrate medium failed to show reduction to ammonia.

Changes in Litmus Milk. Changes in litmus milk at 26 degrees C. were very slow. All cultures produced alkalinity without changing the consistency of the milk.

Peptonization started after one month, and by the end of six weeks the medium became golden - yellow and serum-like.

Indole Production. Indole production was studied by culturing in bacto - tryptone broth, pH_h 6.8, for 24 hours at 26 degrees C. and at 37 degrees C. Kovac's modification of the Ehrlich test was chosen. It is easy to perform and shows a bright red color in about one minute in positive cases. The reagent consists of p-dimethylaminobenzaldehyde 5 gm, amyl alcohol 75 gm, and concentrated hydrochloric acid 25 gm. This reagent did not deteriorate during storage for one year ijn the laboratory.

None of the isolates produced indole at either temperature of incubation.

This failure could not be ascribed to failure since the reagent gave positive reactions with Escherichia coli and Proteus vulgaris [1].

Production of Hydrogen Sulphide. Two tubes of Difco lead - acetate medium were used for each culture. Four stabs were made in each tube between the solid medium and the glass wall, thus giving eight replications. Incubation was for 8 days at 26 degrees C. Blackening of the medium along

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1) A culture of Proteus vulgaris showed a strongly positive reaction after one day at 26 degrees C., despite the fact that this species is described in Bergey's Manual(5) as "weakly positive" .

a stab was taken as an indication of hydrogen sulphide formation.

All cultures were positive for hydrogen sulphide.

Starch Hydrolysis. Difco starch agar slants were inoculated from young broth cultures and incubated for four days at 26 degrees C. Lugol's iodine was used as the reagent.

None of the cultures hydrolized starch.

Methyl-Red Test. This test is merely a confirmation that glucose is acidified, and was performed as recommended[1].

All cultures produced acid; 15 producing more than others.

Vosges-Proskauer Test. This test is based upon the production of acetyl-methyl carbinol from glucose.

Results were variable. Twenty cultures produced positive, 14 weakly positive and 4 negative results.

1) Manual of Methods for Pure Culture Study of Bacteria. Society of American Bacteriologists, Committee on Bacteriological Technique, Geneva, N.Y.

Catalase. The presence of this enzyme was determined by dropping hydrogen-peroxide on surface colonies of the cultures and watching for bubbles of liberated oxygen.

All cultures liberated oxygen and may be considered catalase positive.

Action on Fats. The action of six typical cultures on ten pure tri-glycerides and two natural fats was tested by the method of Hammer and Collins (17).

All cultures attacked tri-proprionin, producing a clear zone 4 to 10 mm wide in one week. They attacked tri-butyrin more slowly; the clear zone being only 1 to 2 mm wide at the end of two weeks. They produced no change in the other fats, even in three weeks.

Chromogenesis. Young growth on solid media produced at 26 degrees C. a pale, creamy-yellow pigment, which changed to golden-yellow on ageing. The color was somewhat darker at 37 degrees C.

Fluorescence. Three media were used to detect fluorescence:

a) 0.5% sucrose nutrient broth

b) asparagine 1%, $MgSO_4$ 0.18%, K_2PO_4 0.03%, KH_2PO_4 0.03%, pH 6.1.

c) asparagine 0.3%, $MgSO_4$ 0.1%, K_2PO_4 0.05%, pH 7.2 (Georgia and Poe's medium).

The cultures were inoculated into these media, incubated for 6 days at 26 degrees C. and tested by exposure to ultra-violet light in a dark room.

None of the cultures fluoresced.

Oxygen Relations. Growth was uniform from top to bottom in stab-cultures in several solid media at 26 degrees C. and at 37 degrees C. Nutrient agar roll-tubes at 26° C. showed growth only near the top. Nutrient broth, while uniformly turbid, had a somewhat better growth near the surface.

Temperature Relations. Growth took place over a wide range of temperature: 6, 26 and 37 degrees C. The optimum temperature seemed to lie between 26 and 37 degrees C.

Description of Two Cultures differing from Type I

Two cultures, one from wheat and one from oats, not considered in the foregoing, differed from Type I as follows:

nutrient broth - thicker membrane, more alkaline
gelatin - not liquefied
litmus milk - alkaline, consistency unchanged
maltose agar - no zooglea
incubation at 37 degrees C. - weaker growth in nutrient broth and tryptone broth

They differed from each other in the following respects:

The culture isolated from wheat did not reduce nitrate to nitrite and attacked tri-proprionin vigorously, clearing 25 mm in one week. It also attacked tri-butyrin more strongly than did Type I.

This organism was identified as Flavobacterium turcosum (Zimmermann) Bergey et al.[1)]

The culture isolated from oats reduced nitrate to nitrite and showed the same weak action on tri-proprionin and tri-butyrin as Type I. This culture does not appear to belong to any described species.

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1) Pseudomonas turcosa (Zimmermann) Migula in the 6 th edition of Bergey's Manual.

Discussion on yellow-pigmented bacteria on plants

Many investigators have reported finding yellow-pigmented bacteria on higher plants and plant-parts, as shown in Table 16.

Table 16

Previous Reports on yellow-pigmented Bacteria on Plants

Year	Investigator	Reference	Habitat
1888	Beijerinck	3	red clover
1890	Frank	4	various plant roots
1899	Winkler	37	potato skin, plum leaves
1902	Chraszcz	12,24	hull of barley seeds
1903	Burri	9	healthy green plants, plant products
1904	Duggeli	12	healthy plants, seeds
1906	Beijerinck et al.	4	calyptra slime of root tips
1907	Huss	20	clover hay, oat seeds
1910	Zikes	17,24	roots and seeds of barley
1914	Wigger	24	bran
1917	Kursteiner	24	straw,black straw,mill dust
1918	Morgenthaler	26	green malt,stored wheat
1923	Fred et al.	15	green plants,sweet corn
1927	Hummer	19	barley seeds and rootlets
1929	Woller	38	plants,seeds,cereals
1930	Grapengeter	24	green plants
1931	Huttig	24	green plants, air of cow barns
1932	Keipper et al.	24	cabbage leaves
1936	Mack	24	green plants
1938	Bojko	6	stored rye seeds
1939	Amos	2	stored wheat seeds, flour
1941	Kretovich et al.	22	stored wheat seeds
1946	James et al.	21	stored wheat seeds

In this study most of these reports will not be considered further. Some of them present more details and may be used for purposes of comparison. Summarized descriptions of the findings of Duggeli, Mack and Huss are presented in Table 17, along with similar information on Type I.

Table 17. Comparison of Type I with yellow-pigmented bacteria isolated from higher plants and seeds by others

	Type I	Mack	Duggeli	Huss
Number of Isolates	38	28	6	1
Shape	rod	rod	rod	rod
Arrangement	single	single	single-double	single, often 2
Size (microns)	1.6-2.7x0.8	2-3 x 0.7	1.5-2 x 0.7	0.7-2 x 0.6
Flagella	1-2 polar	mostly 2 polar	unsuccessful	1 polar
Gram	negative	negative	negative	negative
Spores	none	none	none	none
Motility	positive	positive	positive	positive
Pigmentation young	lt.yellow	very yellow	golden yellow	lt.yellow
old	golden yellow			deep yellow
Gelatin Liquefaction	positive	positive	positive	positive
Indole Production	negative	negative	positive	positive
Nitrate Reduction				
to Nitrites	positive	positive	positive	positive
to Ammonia	negative	negative	-	-
Litmus Milk	slow pept.	unchanged	unchanged	slow pept.
H_2S Production	positive	negative	-	positive
Vosges-Proskauer	neg. to pos.	positive	-	-
Catalase	positive	positive	-	-
Nutrient Broth (pH)	alkaline	unchanged	acid	alkaline

(continued on the next page)

Table 17 continued

Comparison of Type I with yellow - pigmented bacteria isolated from higher plants and seeds by others

	Type I	Mack	Duggeli	Huss
Fermentation Reactions:				
Xylose	acid	acid	-	acid
Arabinose	acid	acid	-	acid
Rhamnose	acid	not acid	-	-
Glucose	acid	acid	-	acid
Mannose	acid	acid	-	-
Fructose	acid	-	-	-
Galactose	acid	acid	-	-
Sucrose	acid	acid	-	acid
Maltose	acid	acid	-	-
Lactose	acid	rarely acid	-	not acid
Raffinose	unchanged	-	-	acid
Starch	rarely acid	acid	-	-
Inulin	not acid	acid	-	-
Dextrin	not acid	acid	-	-
Salicin	acid	-	-	-

It may be noted from Table 17 that there exists a close agreement with regard to the morphological characteristics of these bacteria. Sometimes double rods or short chains were reported, which might have been due to failure to separate immediately after cell division.

Also, of particular interest is the unanimity with regard to type and number of flagella. The observation that only one to two polar flagella exist is important for the proper classification of this species.

The problem of physiological characteristics cannot be disposed of so easily. There is disagreement with respect to chemical changes in some substances, while in other cases the data are not included in the report. It is complicated by the fact that different investigators did not use the same methods. Accordingly, a more detailed report on physiological characteristics will be presented.

Fermentation Tests. Two basal media are in use for testing the fermentation of carbohydrates and pure alcohols. The Committee on Bacteriological Technique[1)] lists beef extract peptone broth to which the fermentable substance is added. Salvin and Lewis (31) pointed out that

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1) See footnote on page 38

proteinaceous media may be utilized by certain bacteria with the formation of ammonia. This would neutralize acid produced from a fermentable substance. Many investigators prefer a basal medium consisting entirely of inorganic salts. In this medium any change in reaction can be ascribed to the fermentable substance added to it. Much evidence of the superiority of a synthetic medium over a carbohydrate broth is to be found in the literature.

The kind of basal medium, the purity and concentration of ingredients, the time of incubation and method of determining the pH (electrometrically or colorimetrically) are some of the factors affecting fermentation results.

Duggeli's original description did not include fermentation results.

Mack determined the pH of four cultures electrometrically in a sugar-phosphate medium. She found that these cultures produced acid in pentoses, hexoses, disaccharides, polysaccharides and the alcohol, mannitol. The reaction remained unchanged in dulcitol, inositol, and glycerine. Mack stated that while most strains did not attack lactose, some did, which she ascribed to "Karstrom's adaptive enzymes".

Huss titrated the acid produced in a carbohydrate peptone broth against 0.25 N sodium hydroxide. He found that

at 20° C. his isolate produced acid from xylose, arabinose, sucrose, raffinose and mannitol. Glucose showed only slight acidity. The lactose medium became alkaline. Inoculated broth without carbohydrates also became alkaline in reaction. At an incubation temperature of 30 degrees C. acid was produced from glucose, while raffinose became alkaline in reaction.

Litmus Milk. Duggeli and Mack reported "litmus milk unchanged". Huss mentioned peptonization after two weeks at 20 degrees C.

Mack incubated her cultures for ten days only, and it is not known how long Duggeli incubated his. Considering the possibility that litmus milk cultures may not have been incubated sufficiently long to produce changes, the statement that "litmus milk remained unchanged" is open to criticism. In this study, litmus milk was peptonized, though only very slowly.

Indole Production. Type I did not produce indole in tryptone broth. Mack, who also used Kovac's reagent, reported that no indole was produced. Duggeli and Huss, on the other hand, reported that indole was produced by their cultures.

One can only speculate as to the causes of the positive reaction reported by these two investigators. Duggeli may

have used Salkowski's method, since all other methods came into use after Duggeli published his description. Kulp (23), 1924, pointed out that Salkowski's method was unreliable, because substances other than indole would yield positive results.

Huss added among other substances a few drops of 10% sulphuric acid when testing for indole. Sandiford (32), 1937, warned that acid-containing reagents may give false positive reactions in cultures tested for indole.

Hydrogen Sulphide Production. Huss used lead acetate paper strips and obtained a positive reaction for hydrogen sulphide. Mack reported that no hydrogen sulphide was formed by her cultures.

Classification

While the foregoing discussion appears to indicate that there are certain differences among the cultures referred to, it seems evident that they are not of sufficient importance to justify placing them in different species. Accordingly, it may be claimed that these organisms and the cultures referred to as Type I belong to one species.

It appears that six different names have been given to this species. None of these names has been accepted as final

by subsequent investigators and its proper classification has yet to be found.

Beijerinck (3), 1888, isolated from red clover a motile, weakly gelatin-liquefying, brownish, diplococcus-like "Faulnissbacillus". Its colonies resembled Proteus. He did not describe it adequately, but proposed a name, Bacillus anglomerans.

Winkler (37, 1899, obtained bacteria in pure culture from plum leaves. They formed "beautiful yellow colonies" on gelatin plates, and zooglea on wort gelatin. He named it Bacillus mesentericus aureus, because he thought it was similar to Bacillus mesentericus fucus Flugge. Unfortunately, he did not describe the organism. Chrzaszcz (24), 1902, used the same name for bacteria isolated from the hull of barley seeds; so did Burri (9), 1903, who considered them to be the predominant vegetative form on higher plants, occurring sometimes in pure culture.

Duggeli (12), 1904, was the first to publish a description of the species. He named it Bacterium herbicola aureum. This name has persisted to the present time, but has been shortened to Bacterium herbicola to avoid the trinomial.

Beijerinck (4), 1906, referring to the Bacillus herbicola of Burri and Duggeli (note the different name) claimed

priority for his name, Bacillus anglomerans. This claim must be considered invalid according to the rules for nomenclature accepted at the present time.

Huss (20), 1907, isolated from clover hay and oat seeds (tested for the presence of milk-souring bacteria) a yellow pigmented, polar flagellated, motile rod. He described it and placed it in the genus Pseudomonas on account of its flagellation, and gave it the name Pseudomonas trifolii.

Mack (24), 1936, placed Bacterium herbicola in the genus Flavobacterium in accordance with Bergey's system of classification, but disregarded purposely the flagellation of this organism. She concluded that Huss' Pseudomonas trifolii and Wolff's Bacterium trifolii were identical with Flavobacterium herbicola.

The name Flavobacterium herbicola was used by Bojko (6),1938, in Soviet-Russia, but Kretovich and Rautenshtein (22), 1941, also in Soviet-Russia, referred to this species as Bacterium herbicola.

Flavobacterium herbicola is listed in Bergey's manual (5), 1939, as a synonym for Pseudomonas trifolii. This species is described in appendix II of the Bacteriaceae which contains bacteria "that probably should be place in some of the genera included in the Family Pseudomonadeceae".

In attempting to classify this species two questions must be answered: to what genus does it belong and what name is to be accepted as valid. The latter question will be dealt with first.

It appears that Duggeli was the first to publish a description that could be considered adequate for this species, and to accompany the description with a name, Bacterium herbicola. The species name herbicola should accordingly stand.

The problem of genus cannot be settled so easily. The genus Bacterium has been relegated to a position of little significance in the present system of classification, including a somewhat heterogenous group of types. Ultimately, it will not receive genus recognition.

Mack's choice of the genus Flavobacterium was unfortunate. Yellow pigmentation was unduly stressed and flagellation ignored. According to Breed (8), 1945, the genus Flavobacterium has been placed in the family Achromobacteriaceae. in Bergey's latest edition (1947). Motile organisms in this family have peritrichous flagella. Huss and Mack reported that these bacteria have one to two polar flagella; a finding which was confirmed in this study.

The logical place for this species, on the basis of flagellation appears to be in the family Pseudomonadeceae.

Elliott (13), 1943, suggested that this family should include the genera: Pseudomonas, Phytomonas and Xanthomonas.

Xanthomonas is a new genus proposed by Dowson (11), 1939. It includes organisms which are rod-shaped, gram negative, polar flagellated and yellow-pigmented and which do not produce a water soluble pigment.

The description of this genus applies very well to Type I and it is proposed that the species be included in this genus. In this case, the name of the species would have to be changed to

Xanthomonas herbicola (Burri et Duggeli) comb. nov.

Description of Type II

Fifty-six bacterial cultures were isolated from nutrient agar plates prepared from wheat, flax, barley, oats and sweet corn seeds. Fifty-two cultures were sufficiently alike to be grouped together and will be considered to be Type II. The four cultures which differed from Type II will be discussed in a separate sub-section.

The methods used were those described under Type I.

Morphology

The organisms appeared as rods with rounded ends, averaging 1.6 to 0.8 microns when stained with 0.5 per cent safranin. On negative nigrosin mounts they measured up to 2.7 microns in length and 0.9 microns in width. They occurred singly or in short chains. All cultures were motile. Most rods had one or two polar flagella, though some had polar tufts. They were gram negative and had neither spores nor capsules.

Cultural Characteristics

Nutrient Agar - colonies whitish-transparent, circular, entire, convex, glistening, smooth, average diameter 3.8 mm (64 measurements), medium not colored.

Several cultures produced two types of colony. The R-type colonies were whitish-transparent, dentate, irregular, flat, cracked or wrinkled, and slightly larger than S-type colonies, which conformed with the former description.

Nutrient Agar - slant cultures whitish-transparent, abundant, fliform, entire, flat, glistening, smooth. Some cultures produced a green color in the medium.

Nutrient Broth - slight turbidity after 24 hours at 26 degrees C., later becoming strong and uniform, pellicle after a few days in some cases, sediment gray and viscid. Many cultures produced a green color.

Upon incubation all cultures produced an alkaline reaction. After one week the pH was raised from 7.9 to that of 8.5 to 10.6 in different cultures.

Gelatin - liquefaction rapid, infundibuliform, sediment heavy, white. Most cultures liquefied more than half of the medium in one week. The medium was not colored.

Wort Agar (pH 5.0) - three cultures tested on this medium failed to grow.

Physiology

Fermentations. Acid production was studied in Elrod and Braun's medium to which was added one per cent of the

sugar to be tested. Cultures were incubated for 5 days at 26 degrees C. Results are summarized in Table 18. Detailed results are listed in the appendix (Table 71).

Table 18

Changes in pH produced by 52 cultures (4 sugars)

Carbohydrate	Check	Range	Aver. Difference [1]
Glucose	6.25	3.10-6.70	-1.894
Sucrose	7.35	4.50-7.70	-0.500
Lactose	6.25	5.90-6.80	-0.012
Salicin	7.30	7.10-7.50	-0.057

1) calculated by taking the arithmetic average of the differences of 52 pH readings from the average of 5 uninoculated checks per sugar

Table 18 shows that the cultures varied considerably in their ability to produce acid from sugars. Most cultures produced acid from glucose and sucrose, but only a few produced it in lactose and salicin media.

Nitrate Reduction. None of the cultures reduced nitrate to nitrite. The addition of zinc dust to all cultures produced a red color, indicating that the nitrate was still present in the medium. In the same test, cultures of Pseudomonas aeruginosa, Pseudomonas fluorescens, Pseudomonas fragi and Pseudomonas mucidolens reduced nitrate to nitrite and gaseous nitrogen.

The addition of Nessler's reagent to a replicate of each culture in the potassium nitrate broth (0.1%) failed to show reduction to ammonia.

Indole Production. None of the cultures produced indole.

Starch Hydrolysis. None of the cultures hydrolized starch.

Hydrogen Sulphide Production. All but six cultures produced hydrogen sulphide.

Changes in Litmus Milk. All cultures produced alkalinity and peptonized the milk. Some cultures peptonized rapidly, while others completed peptonization by the end of four weeks. Different cultures discolored the medium yellow, green and blue. pH determinations on these cultures showed that the changes in color were not associated with changes in pH.

Action on Fats. All of the twenty-four cultures tested attacked seven tri-glycerides and the two natural fats, cotton seed oil and linseed oil. The only tri-glycerides not attacked were tri-acetin, tri-myristin, and tri-stearin. Tri-propionin was most strongly and tri-butyrin somewhat less strongly affected.

Temperature Relations. The cultures had an optimum

temperature of 26 degrees C. They were sensitive to high and low temperatures. At 37 degrees C. only four cultures grew. At 6 degrees C. five different cultures developed.

Chromogenesis. The color of colonies and streaks on nutrient agar was similar to the substratum, indicating transparency. Occasionally, a whitish-greyish pigment was formed.

Pyocyanin Production. The production of this pigment was tested by two methods. One consisted of shaking nutrient broth cultures with chloroform. The presence of pyocyanin in the medium is indicated by the formation of a blue precipitate. The other method consisted of streaking cultures on Gessard's medium in duplicate and incubating one set at 26 degrees C. and the other at 37 degrees C. The presence of pyocyanin is indicated by a green color which becomes brown and even black upon prolonged incubation.

None of the cultures produced a blue precipitate or a green color at either temperature of incubation.

Fluorescence. Fluorescence was tested by exposing cultures grown in Georgia and Poe's medium at 26 degrees C. to ultra-violet light in a dark room.

All cultures showed fluorescence.

The cultures did not develop fluorescence at the same rate. Some fluoresced after incubating one day, while

others did not exhibit this characteristic until three days. After longer incubation different colors developed, ranging from yellow to blue. This difference in color did not appear to be related to differences in pH at the time readings were made. This was brought out in an experiment designed to determine the relationship between color development and pH. Two tests were made one after incubating for 5 days and a second after 14 days incubation. The results are presented in Table 19.

Table 19

Relation between Color in ultra-violet light and pH

5 Days		
Color in ultra-violet light	No. of Cultures	Average pH
bright yellow-green, more yellow than green	6	9.35
bright green-blue, more green than blue	2	9.15
bright blue-green, more blue than green	9	8.82
bright uniformly blue	6	9.30

14 Days			
Color in ultra-violet light	No. of Cultures	Aver. pH	Color in Daylight
bright green	7	8.6	bright green
dull green	7	8.6	pale green
blue-green	7	8.7	pale green
blue	3	8.6	pale grey

The following experiment was carried out to determine whether or not the color exhibited in ultra-violet light was a constant characteristic for a given isolate under a standard set of conditions.

For this study four cultures which had been incubated

for two weeks at 26 degrees C. were selected. Two showed strong and uniform blue, one green and the other yellow fluorescence. Each culture was plated on nutrient agar and incubated at 26 degrees C. Ten colonies were picked from each plate and transferred separately to Georgia and Poe's medium, incubated for two weeks and then exposed to ultra-violet light. The following observations were made:

Blue Fluorescent culture No. 1	yielded 2	blue fluorescent cultures
	8	non-fluorescent cultures
Blue fluorescent culture No. 2	yielded 1	blue fluorescent culture
	9	non-fluorescent cultures
Green fluorescent culture	yielded 1	blue fluorescent culture
	9	green fluorescent cultures varying in color
Yellow fluorescent culture	yielded 1	blue fluorescent culture
	9	green fluorescent cultures varying in color

The four cultures were incubated two weeks longer and the

experiment was repeated. The following results were obtained:

Neither of the two blue fluorescent cultures, nor the yellow fluorescent culture produced subcultures that fluoresced.

The green fluorescent culture yielded one blue fluorescent culture and 9 yellow to green fluorescent cultures.

The results of this experiment make it obvious that fluorescence is not a fixed characteristic of a given isolate. A culture may dissociate into some sub-cultures that fluoresce with a characteristic color, some that have other colors and some that do not fluoresce. Furthermore, even in cultures that fluoresce, it appears to be a transient characteristic that cannot be relied upon for identification purposes.

Description of Four Cultures differing from Type II

Two non-motile cultures, otherwise identical with Type II, were isolated from seeds of oats and flax, respectively.

Only one species is listed in Bergey's manual (5), 1939, under:

III. Non-motile rods.

A. Gelatin liquefied

a. Milk not coagulated

b. Nitrites not produced from nitrates

c. Indole not formed

28. Pseudomonas smaragdina.

These two cultures appear to agree closely with this species, except that the optimum temperature was 26 degrees C. and not 35 degrees C., and that no odor resembling jasmin was noticed. The species described in Bergey's manual was isolated from nasal secretions in ozena.

Two chromogenic cultures were isolated from wheat seeds. One was motile and one was non-motile. They were identical in all other characteristics, but differed from Type II in:

chromogenesis - ochre yellow

nutrient broth - no turbidity for the first two days

gelatin - no liquefaction

litmus milk	- consistency unchanged
glucose	- no acid produced
sucrose	- no acid produced
fats	- tri-propionin not attacked the first two days

The non-motile culture appears to be Pseudomonas fluorescens non-liquefaciens. The motile culture resembles the Pseudomonas ovalis group described in Bergey's manual.

Discussion on Fluorescent Bacteria on Plants

Beijerinck (3), 1888, isolated Bacillus fluorescens liquefaciens and Bacillus fluorescens putidus from nodules of leguminous plants. Since then many investigators have reported finding fluorescing bacteria on plants.

Burri (9), Duggeli (12) and Hummer (19) mentioned that Bacterium fluorescens liquefaciens and Bacterium putidum occurred in large numbers on green plants. The last author stated that Zikes found that Bacterium fluorescens and Bacterium herbicola grew symbiotically on barley seeds.

Mack (24) found that 48 per cent of the total bacterial population on plants consisted of types which fluoresced and liquefied gelatin, and that 17 per cent consisted of types which fluoresced, but did not liquefy gelatin. She quoted Huttig, Henneberg, and Grossengeter, who, independently, stressed the regular occurrence of fluorescent bacteria on plants. Mack concluded that Bacterium fluorescens, Bacterium putidum and Bacterium punctatum were regularly associated with Bacterium herbicola.

Morgenthaler (26) reported that fluorescent bacteria were rare on musty grain.

Woller (38) stated that cereal seedlings did not harbour fluorescent bacteria in certain years, while in other years they constituted the major portion of the population.

Taxonomy

Type II does not appear to belong to any species of Pseudomonas listed in Bergey's manual.

By introducing a new sub-section bb. under the "milk not coagulated" section, a place for it would be provided. This is shown schematically as follows:

I. Motile rods, polar flagella

A. Gelatin liquefied

a. Milk coagulated; peptonized

aa. Milk not coagulated

b. Nitrites produced from nitrates

c. Indole not formed

bb. Nitrites not produced from nitrates

c. Indole not formed nov. sp.

aaa. Milk unchanged

etc.

The name Pseudomonas herbosa (meaning abounding on herbage) is suggested for the new species.

Part III

MICROORGANISMS ON GROWING PLANTS

The microflora of wheat plants was investigated to obtain information about numbers and types of organisms on various parts of the plant. The main objective was to determine whether the numbers and types of microorganisms remained constant or changed as the plant developed. Particular attention was given to the two main species of bacteria found on seeds and to the reproductive structures, since it is not known how these bacteria reach the developing seed.

Through the courtesy of Dr. R. Peterson, Dominion Laboratory of Plant-breeding, Winnipeg, two plots of wheat, variety Redman, were set aside for investigations in the summer of 1946. Additional experiments were carried out with seedlings grown under controlled conditions in the greenhouse during the following winter.

Field Experiments

Plants in different stages of development were used. They were handled aseptically during their removal from the field and subsequent treatment. They were cut just above the ground, put into sterile containers and taken immediately to the laboratory. When plants were still young only the

leaves were tested. A part in the center of the leaf was cut out, measured and macerated in a mortar. In older plants the leaves became too hard to be crushed, and were left intact. Counts were expressed per one square centimeter of surface area.

After the plants developed stems, portions of these were also tested. Stems were left intact, because it was found difficult to macerate them. Counts were expressed per one centimeter length of stem.

Heads were also included in the experiments. In some cases, heads which had not yet emerged from the sheath were used. The heads were either left intact or broken up into individual florets.

Leaves and stems were put into 10 cc of sterile water, shaken by hand 25 times, and one cubic centimeter was used for plating purposes. It was found that the small amount of plant juice when mixed with the sterile water and then with nutrient agar did not influence the pH of the medium.

Heads were put into 100 cc of sterile water, containing gravel, and shaken for half an hour on a mechanical shaker. One cubic centimeter of the wash-water was then used.

Nutrient agar was used for bacteria; acidified

Czapek's agar for fungi and yeasts. Incubation was for 5 days at 26 degrees C.

1. Plants in the Pre - heading Stage

The seed used for this study contained 470 000 bacteria, 32 500 fungi and 17 500 yeasts per gram. Both species of bacteria, found regularly on other samples of grains, were present in large numbers, Type I being more numerous than Type II. Seeding was done on April 13, and the first seedling samples were tested on May 8, when the plants were about 10 cm tall. The results are presented in Table 20.

Table 20

Average Number of Microorganisms on Plants in the Pre - heading Stage

Date Sampled	Parts used	Repl.	Bacteria	Fungi	Yeasts
May 8	Leaves	3	41	7	none
May 21	Leaves	3	27	-	-
May 27	Leaves	4	21	3	none
June 3	Leaves	2	6	16	none
June 14	Leaves	2	31	28	none
June 14	Stems	2	128	13	none

- not plated for fungi

The data show that the numbers of microorganisms on these plants were small and that no important changes in numbers took place. Colonies resembling those of the two main types of bacteria found on seeds were absent. The bacteria which were present on the plants consisted of a variety of types. Some formed spreading colonies, others various brightly pigmented colonies, consisting of micrococci and others grey colonies containing long, stout rods. The fungi likewise represented different types. This microflora was similar to that usually obtained from aerial contamination.

2. Plants in the Heading Stage

Plants in the field do not grow at the same rate. At the time when some are headed other heads are still enclosed in the sheath. This difference in development was made use of to compare headed and non-headed plants. Counts were again expressed per square centimeter of leaf area and per centimeter length of stem. The results of this comparison are listed in Table 21.

Table 21

Number of Microorganisms on Headed and Non-Headed Plants

Date Sampled	Parts used	Headed Plants Bact.	Fungi	Non-Headed Plants Bact.	Fungi
June 22	Flagleaf 1	1 438	18		
	Flagleaf 2	108	15		
	Middle Leaf	160	27		
	Stem below Head-1	665	45		
	Head intact	11 150	60		
	broken up	495	315		
June 28	Flagleaf 1	55	77	223	97
	Flagleaf 2			16	154
	Middle Leaf	1 235	63		
	Stem below Head 1	60	69		
	Stem below Head 2	49	-		
	Stem below Flagleaf	3 129	57		
	Head Intact	2 050	7 000		
	broken up	10 550	9 000		
July 10	Flagleaf	4 300	-	300	-
	Sheath (entire)			245	-
	Stem below Flagleaf	496 000	-		
	Heads (Three) over	500 000	-		

Table 21 shows that numbers of bacteria on the same part of different plants fluctuated widely and that headed plants harboured more bacteria than non-headed plants. The number of bacteria on the heads was surprisingly large when compared to other parts of the plant.

The number of fungi on the heads was also larger than on any other part, but was not greatly influenced by the fact that the plant had headed.

Type I was found for the first time on June 28, on a flagleaf of a headed plant, while it was not found on flagleaves of plants that had not yet headed. Almost all colonies prepared from a head which was removed from the sheath consisted of Type I.

Type II was found for the first time on July 10, on a head which had emerged from the sheath.

3. Plants in Four Stages of Development

Taking plants from the field on successive dates has the disadvantage that some plants are longer exposed to climatic conditions than others. To eliminate this difference in length of exposure to weather, four plants in different stages of development were selected on one date.

Plant I had the head enclosed in a sheath which was not split

Plant II had the head enclosed in a sheath which was slightly split

Plant III had the head enclosed in a sheath which was wide open

Plant IV had the head emerged from the sheath which had become part of the stem.

The first three plants were immature, thin, and spindly. Since the growing season was almost over it appeared doubtful whether these plants would have developed normally.

The numbers of bacteria are listed in Table 22.

Table 22

Number of Bacteria on Various Parts of Plants In Different Stages of Development

		Flagleaf sq. cm	Sheath entire	Head entire	Leaf next to Sheath sq. cm
Plant	I	86	833	130	469
	II	88	22 041	1 200	
	III	55	636 735	56 000	
	IV	5	340[1)]	230	

1) sheath, now part of the stem

The data show again that the number of bacteria fluctuated greatly, from part to part on one plant, as well as between plants.

Type I occurred in large numbers on Plant III, but was absent on the other plants. Type II was not isolated from any of the plants.

4. Over-ripe Plants

Plants in the two plots were left standing long beyond the date on which they would have been harvested ordinarily. These plants, therefore, were "over-ripe". Numbers of bacteria in Table 23 refer to entire heads and leaves, while those of stems are expressed on the per centimeter of length basis

Table 23

Number of Bacteria on Five Over-ripe Plants

		Heads	Flag-leaf	below heads	Stems below flagleaves
Plants	I	3 000 000	t.n.[1)]	t.n.	40 000
	II	5 600 000	t.n.	t.n.	t.n.
	III	1 700 000	t.n.	2 400	t.n.
	IV	30 000 000	t.n.	54 000	t.n.
	V	13 300 000	t.n.	2.200	t.n.

1) t.n. means too numerous to count in the 0.001 dilution

Such high counts were not anticipated. Consequently, a sufficiently high dilution was not used to permit an estimate in many of the tests.

Practically all the colonies appeared to be Type I or Type II. Probably the weathering to which these plants were exposed caused a large increase in these types.

Greenhouse Experiments

When in the field - experiments plants were tested for the first time they had already reached a height of about 10 cm. In order to obtain information with regard to plants immediately after emergence and in the early stages of development, seedlings were grown in the greenhouse. The main purpose of these experiments was to find out whether the two main species of bacteria were present on the young seedling.

Carefully selected, healthy kernels of the variety Renown were planted in a mixture of three parts soil, one part sand and two parts manure. The plants were cut in the one-leaf, two-leaf and three-leaf stage, put into 10 cc of sterile water, containing gravel, and shaken for half an hour on a mechanical shaker. One cubic centimeter of the wash-water was plated in duplicate on nutrient agar and on acidified Czapek's agar, respectively. Incubation was for six days at 26 degrees C.

1. Microflora on Young Seedlings

Ten seedlings in the one-leaf, two-leaf and three-leaf stage were used to determine the numbers on young seedlings. The average number of bacteria, yeasts and fungi is listed in Table 24.

Table 24

Number of Microorganisms on Ten Young Seedlings

Stage	BACTERIA Aver. No. per Seedling	Range	FUNGI Aver. No. per Seedling	Range	YEASTS Aver. No. Per Seedling
One-leaf	1 040	0- 7 450	45	25-107	none
Two-leaf	2 925	350- 8 900	318	90-960	none
Three-leaf	13 600 [1)]	3 300-25 600	1 630	400-560	80

1) two seedlings had colonies too numerous to count

These data show that there was a steady increase in numbers of microorganisms as the seedlings grew older.

2. Type I and Type II on One-leaf Seedlings

In the foregoing experiment, bacteria of Type I and Type II were found on seedlings in the one-leaf stage. As the seedlings grew older these two types decreased in numbers and were replaced by a variety of types. Spreading colonies on plates from three-leaf seedlings caused great discrepancies in counts on replicate plates e.g. 175 and 55, and 118 and 70. This might have been due to antibiosis on part of the spore-formers.

Eighty one-leaf seedlings were tested for the presence of Type I and Type II. The following results were obtained:

5 seedlings did not develop any colonies on plates

35 seedlings had one to a few yellow colonies per plate, most of which did not resemble those of Type I.

45 seedlings had fluorescent bacteria. All surface colonies on plates from ten seedlings fluoresced when cultured in 0.5 per cent sucrose nutrient broth for 5 days and exposed to ultra-violet light.

21 of the above seedlings showed the presence of both types of bacteria.

3. Microflora of the Second Growth of Clipped Seedlings

The seedlings which were clipped just above the ground developed a second growth. When the plants were about 7 cm tall they were again cut and tested as before.

For this experiment 25 seedlings were used. Twenty-three of these produced mostly or only fungi on nutrient agar plates. On some plates these fungi belonged to one species only, while on others there were a few species. Two seedlings showed mostly or only bacteria which did not belong to Type I or Type II.

It seems that bacteria were of little importance on these seedlings, but that a definite fungal flora had established itself, consisting of few types only. These types

constitute in all probability, secondary wound invaders.

The results appear to indicate that seedlings harbour an epiphytic population of bacteria which is lost when the first growth is removed by cutting.

Microflora of Reproductive Structures of Wheat Plants

It is not known by what mechanism epiphytic bacteria reach the developing seeds. In order to determine whether these bacteria may be found on the reproductive structures of wheat plants the following tests were carried out.

The method consisted of removing the ovary and anthers from the floret and dropping these parts either separately or together into tubes containing nutrient broth. In 1946 a 0.5 per cent sucrose - nutrient broth medium was used, while in 1947 ordinary standard nutrient broth was used. Incubation was for two weeks at 26 degrees C., to give slow growing bacteria a chance to develop. Turbidity was taken as an indication of bacterial growth. Tubes which remained clear were then incubated at 37 degrees C. None developed turbidity at 37 degrees C. Nutrient broth showing signs of bacterial growth was streaked on nutrient agar plates to determine the types of bacteria. The tubes which showed fungal growth were discarded.

1. Unfertilized Wheat Plants (1946)

Five heads which were still enclosed in the sheath were used.

One hundred tubes were inoculated with reproductive structures from various florets.

Ninety - seven of these remained clear even after two weeks of incubation.

2. Unfertilized Wheat Plants (1947)

Seventy - five tubes were inoculated with reproductive structures from various florets.

Fifty - seven of these remained clear after one week of incubation.

Most colonies on plates prepared from the 18 tubes which showed growth were identical in appearance. They contained spore - forming rods.

3. Fertilized Wheat Plants (1946)

Seven tubes were inoculated with fertilized reproductive structures; the presence of swollen ovaries and pollens being accepted as indicative of fertilization.

Four of these remained clear even after two weeks of incubation.

Large, spreading colonies developed on plates prepared from one tube showing turbidity, while many transparent colonies developed on plates prepared from another tube.

Thirty-nine fertilized ovaries which had the anthers still attached to them were put into 0.5 per cent sucrose nutrient broth. Incubation was for two weeks at 26 degrees C.

Eleven of these remained clear,

Sixteen contained fungi which grew on the surface, and

Twelve contained bacteria.

Eight of the 12 bacterial cultures were plated on nutrient agar.

Two of these produced colonies that were uniformly, deeply yellow, resembling Type I.

Two produced uniformly whitish colonies, resembling Type II.

Two colonies contained a mixture of Type I and Type II.

One of the eight formed whitish, large, irregular colonies which did not fluoresce.

The remaining culture which developed from a tube containing only anthers produced colonies that were ochre to orange in color, convex, smooth, circular, glistening and entire.

The preceding results appear to indicate that

1. Unfertilized reproductive structures are sterile or harbour chance contaminants -- mainly spore-formers.
2. At least a portion of the fertilized structures harbour bacteria regularly found on the seed.

Discussion

Burri's theory that the bacterial flora of a plant is the result of multiplication of certain species on the surface of the plant has been subsequently supported by experimental evidence. Several investigators (12, 15, 21, 24, 26, 34) found that certain species occurred regularly on green plants and seeds, but only very rarely in soil or in the air.

Woller (38) held the view that the epiphytic bacteria represented an assortment of frugal air and soil organisms. He should not have used the term "epiphytic" for these organisms.

Results of experiments with wheat seedlings and wheat plants in this study confirm both views. Soil and air types were found on young plants in larger numbers than were the two main species of bacteria. These contaminants of young plants did not seem to multiply on the plant. On the contrary, their numbers declined steadily, while those of the two species increased until they reached huge proportions. Multiplication was indicated by the enormous numbers of Xanthomonas herbicola and Pseudomonas herbosa that were on over-ripe plants.

The mechanism by which these bacteria reach the seedling from the germinating seed is a matter for speculation.

Wallin (36), 1946, traced the path of the plant-pathogen Xanthomonas translucens var. cerealis from seed to seedling. He found that the pathogen persisted in the intercellular spaces of the coleoptile from where it infested the first foliage leaf. Wallin concluded that "there was no evidence that the bacteria invade the seedling except through the coleoptile." Simmonds (34), 1947, found that "the abundance of the bacteria on the surface of the coleoptile originating from a seed carrying surface bacteria, indicates, it is believed, a definite surface spreading characteristic of the bacterium concerned." That the epiphytic bacteria do not seem to penetrate the tissues of the coleoptile, as pathogens do, appears to be indicated by the fact that the non-fertilized reproductive structures studied were found to be bacteriologically sterile. The floral primordium forms in the tissues of the coleoptile, and would become contaminated by bacteria living within the coleoptile.

The following theory is proposed to explain the cycle by which epiphytic bacteria reach the seedling from the germinating seed and how they reach the developing seed from the plant. A migration takes place from the surface of the seed to the surface of the coleoptile. From there they move to the growing point, which later becomes the head of

the plant. It is inevitable that some bacteria should reach the first leaf. This explains why both types of bacteria were found on the first leaves, but not on the second and third leaves of the seedling. The bacteria remain on the slowly developing head, but do not enter the tightly closed florets and do not invade the reproductive structures. As soon, however, as fertilization takes place the bacteria move into the florets, and on the reproductive organs. It is in this manner that they invade the developing seed.

It is believed that physiological changes in the growing point attract bacteria and favour their existence there until the plant reaches maturity. Physiological changes which lead to maturity of the plant and to fertilization seem to favour the rapid multiplication of epiphytic bacteria on the head. From there they migrate to all parts of the plant. This may explain why over-ripe plants appear to be covered by millions of bacteria belonging to these two species.

The role of epiphytic bacteria is obscure at present. Fred, Peterson and Anderson (15), 1923, thought that inhabitants of living plant tissues may play an important role in nature. These authors were primarily interested in the

ability of these organisms to ferment pentoses, particularly arabinose and xylose. Hummer (19), 1927, concluded that large numbers of Bacterium herbicola on seeds of cereals were a sign of the good condition of the seed, because in musty grain fungi prevailed, while Bacterium herbicola played only a small part. Morgenthaler (26), 1918, expressed an interesting thought when he said:

> It would be worth while to investigate the biological importance of Bacterium herbicola and the epiphytic bacteria. One can assume that the grain kernel, and, perhaps, also other plant parts are protected from damaging organisms under normal conditions by a layer of Bacterium herbicola. (translated)

Simmonds (34), 1947, stated that "...certain bacteria antibiotic to Helminthosporium sativum are commonly found on the surface of wheat seeds and other parts of the wheat plant... It is not beyond probability that antibiotic bacterial flora may play a very important role in respect to disease resistance in general. It may be the host's first line of defense, and more studies along this approach are needed."

SUMMARY

Commercial samples of Canadian flax, barley and oat seeds, collected in 1946 and 1947, harboured an exceedingly large population of bacteria. A gram of grain contained millions of bacteria, but only relatively few fungi. Yeasts occupied an intermediate position. The number of bacterial spores was quite small. Coliform bacteria were found on some samples in very small numbers.

Hulless oats harboured fewer bacteria and yeasts than hulled oats. The same was not true of fungi. From the second to the third year of storage a considerable reduction in numbers of bacteria and yeasts, but not of fungi, took place.

Approximately 80 per cent of the bacteria belonged to two species, which were found on all samples. Thirty-eight yellow-pigmented cultures belonged to one species which was given the name Xanthomonas herbicola (Burri et Duggeli) comb. nov. One culture was identified as Flavobacterium turcosum (Zimmermann) Bergey et al., while another culture could not be identified.

Fifty-two fluorescent cultures belonged to the other main species, which is a new species. The name Pseudomonas herbosa nov. sp. was suggested. Four fluorescent cultures

were identified as follows: one was Pseudomonas fluorescens non-liquefaciens, two were Pseudomonas smaragdina, and one belonged to the Pseudomonas ovalis group in Bergey's Manual.

The microflora on wheat plants growing in the field consisted of bacteria and fungi. Yeasts were absent and were only found on over-ripe plants. Numbers of bacteria were small on plants in the pre-heading stage, but increased greatly after heads appeared. Fully mature and over-ripe plants harboured very large numbers. On young plants a variety of bacterial types was found. On older plants Xanthomonas herbicola and Pseudomonas herbosa became the predominating types. Numbers of fungi were small on all plants studied.

Seedlings grown in the greenhouse harboured both main species of bacteria shortly after their emergence. Numbers of bacteria and fungi increased from the one-leaf to the three-leaf stage of seedlings. Yeasts were found for the first time on three-leaf seedlings.

The second growth of clipped seedlings harboured mostly fungi, belonging to few species only. Bacteria were almost completely absent. No yeasts were found.

Of 175 unfertilized reproductive structures 88 per

cent were sterile. Non-sterile structures harboured mainly spore-forming bacteria.

Of 46 fertilized reproductive structures only 32.6 per cent were sterile. Non-sterile structures harboured mainly bacteria belonging to the two main species.

The following theory is proposed to explain the mechanism by which Xanthomonas herbicola and Pseudomonas herbosa reach the seedling from the germinating seed and the developing seed on the growing plant:

These two species migrate from the surface of the seed to the developing coleoptile and from there to the growing point which becomes the head of the plant. Since unfertilized reproductures are free from these organisms, but harbour them after fertilization has taken place, it is to be assumed that physiological changes during fertilization attract the bacteria to the anthers and ovaries. In this manner the developing seed becomes invaded. Conditions on the growing point and the developing head seem to favour the existence and multiplication of these bacteria.

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APPENDIX

FLAX

Bacteria

(Counts in the Dilution 1:100 000)

Table 25

Average Number on Duplicate Plates for 1946

Grade	Months	Replications			Total of Month
		I	II	III	
C.W.1	April	169	229	233	631
	May	275	190	250	715
	June	38	46	9	93
	July	54	76	42	172
C.W.2	April	1 575	2 005	2 030	5 610
	May	830	1 360	1 320	3 510
	June	172	164	214	550
	July	400	410	545	1 355
C.W.3	April	130	141	138	409
	May	2 290	2 350	2 240	6 880
	June	127	165	84	376
	July	595	470	465	1 530
C.W.4	May	108	123	93	324
	June	20	18	3	41

Table 26

Data for the Analysis of Variance for 1946

	C.W.1	C.W.2	C.W.3	Total of Month	Mean
April	631	5 610	409	6 650	2 216.67
May	715	3 510	6 880	11 105	3 701.67
June	93	550	376	1 019	339.67
July	172	1 355	1 530	3 057	1 019.00
Total of Grade	1 611	11 025	9 195	21 831	
Mean	402.75	2 756.25	2 298.75		

Necessary Difference for Grades = 101.21 [1)]

Necessary Difference for Months = 116.86

	Totals of Replications		
	I	II	III
April	1 874	2 375	2 401
May	3 395	3 900	3 810
June	337	375	307
July	1 049	956	1 052

1) Necessary Difference in all cases at the 5% point

Table 27

Average Number on Duplicate Plates for 1947

Grade	Months	Replications			Total of Month
		I	II	III	
C.W.1	May	650	290	500	1 440
	June	370	310	260	940
	July	180	190	130	500
	August	52	5	6	63
C.W.2	May	600	1 300	1 720	3 620
	June	70	990	670	1 730
	July	320	310	130	760
	August	200	110	210	520
C.W.3	May	730	850	1 130	2 710
	June	160	160	770	1 090
	July	130	170	110	410
	August	22	16	18	56
C.W.4	June	720	580	160	1 460
	July	210	290	390	890

Table 28

Data for the Analysis of Variance for 1947

	C.W.1	C.W.2	C.W.3	Total of Month	Mean
May	1 440	3 620	2 710	7 770	2 590.00
June	940	1 730	1 090	3 760	1 253.33
July	500	760	410	1 670	556.67
August	63	520	56	639	213.00
Total of Grade	2 943	6 630	4 266	13 839	
Mean	735.75	1 657.50	1 066.50		

Necessary Difference for Grades = 211.25

Necessary Difference for Months = 243.94

	Totals of Replications		
	I	II	III
May	1 980	2 440	3 350
June	600	1 460	1 700
July	630	670	370
August	274	131	234

Fungi

(Counts in the Dilution 1:100)

Table 29

Average Number on Duplicate Plates for 1946

Grade	Months	Replications I	II	III	Total of Month
C.W.1	April	6	15	9	30
	May	42	13	11	66
	June	10	8	7	25
	July	9	13	8	30
C.W.2	April	17	21	30	68
	May	25	18	10	53
	June	21	15	20	56
	July	32	30	18	80
C.W.3	April	31	38	96	165
	May	36	70	29	135
	June	38	40	24	102
	July	34	32	42	108
C.W.4	June	43	94	46	183
	July	49	60	62	171

Table 30

Data for the Analysis of Variance for 1947

	C.W.1	C.W.2	C.W.3	Total of Month	Mean
April	30	68	165	263	87.67
May	66	53	135	254	84.67
June	25	56	102	183	61.00
July	30	80	108	218	72.67
Total of Grade	151	257	510	918	
Mean	37.75	64.25	127.5		

Necessary Difference for Grades = 11.76

	Totals of Replications		
	I	II	III
April	54	74	135
May	103	101	50
June	69	63	51
July	75	75	68

Table 31

Average Number on Duplicate Plates for 1947

Grade	Months	Replications			Total of Month
		I	II	III	
C.W.1	May	19	25	10	54
	June	9	11	11	31
	July	16	42	12	70
	August	12	23	14	49
C.W.2	May	25	45	27	97
	June	22	33	22	77
	July	25	14	17	56
	August	67	27	20	114
C.W.3	May	170	155	120	445
	June	28	55	38	121
	July	66	33	81	180
	August	33	43	36	112
C.W.4	June	760	1 270	830	2 860
	July	160	40	40	240

Table 32

Data for the Analysis of Variance for 1947

	C.W.1	C.W.2	C.W.3	Total of Month	Mean
May	54	97	445	596	198.67
June	31	77	121	229	76.33
July	70	56	180	306	102.00
August	49	114	112	275	91.67
Total of Grade	204	344	858	1 406	
Mean	51	86	214.5		

Necessary Difference for Grades = 14.17

Necessary Difference for Months = 14.48

	Totals of Replications		
	I	II	III
May	214	225	157
June	59	99	71
July	107	89	110
August	112	93	70

Yeasts

(Counts in the Dilution 1:1 000)

Table 33

Average Number on Duplicate Plates for 1946

Grade	Months	Replications I	II	III	Total of Month
C.W.1	April	287	360	396	1 043
	May	45	90	100	235
	June	465	375	505	1 345
	July	360	435	145	940
C.W.2	April	220	71	191	482
	May	455	330	100	885
	June	300	640	215	1 155
	July	450	445	330	1 225
C.W.3	April	117	28	51	196
	May	235	440	410	1 085
	June	50	88	105	243
	July	195	385	20	600
C.W.4	May	10	15	40	65
	June	250	30	42	322

Table 34

Data for the Analysis of Variance for 1946

	C.W.1	C.W.2	C.W.3	Total of Month	Mean
April	1 043	482	196	1 721	573.67
May	235	885	1 085	2 205	735.00
June	1 345	1 155	243	2 743	914.33
July	940	1 225	600	2 765	921.67
Total of Grade	3 563	3 747	2 124	9 434	
Mean	890.75	936.75	531.00		

Necessary Difference for Grades = 101.08

	Totals of Replications		
	I	II	III
April	624	459	638
May	735	860	610
June	815	1 103	825
July	1 005	1 265	495

Table 35

Average Number on Duplicate Plates for 1947

Grade	Months	Replications			Total of Month
		I	II	III	
C.W.1	May	290	165	330	785
	June	66	168	308	542
	July	67	91	200	358
	August	18	34	17	69
C.W.2	May	130	350	1 250	1 730
	June	268	485	596	1 349
	July	575	960	525	2 060
	August	152	110	63	325
C.W.3	May	350	3 250	1 425	5 025
	June	28	74	93	195
	July	225	117	75	417
	August	48	55	50	153
C.W.4	June	26	117	130	273
	July	240	37	97	374

Table 36

Data for the Analysis of Variance for 1947

	C.W.1	C.W.2	C.W.3	Total of Month	Mean
May	785	1 730	5 025	7 540	2 513.33
June	542	1 349	195	2 086	695.33
July	358	2 060	417	2 835	945.00
August	69	325	153	547	182.00
Total of Grade	1 754	5 464	5 790	13 008	
Mean	438.5	1 366	1 447.5		

Necessary Difference for Months = 132.19

	Totals of Replications		
	I	II	III
May	770	3 765	3 005
June	362	727	997
July	867	1 168	800
August	218	199	130

BARLEY

Bacteria

(Counts in Dilution 1:100 000)

Table 37

Average Number on Duplicate Plates for 1946

Grade	Months	Replications I	II	III	Total of Month
Two Row C.W.1 Dil. 1:1 000	April	83	35	67	185
Two Row C.W.2 Dil. 1:10 000	May	337	65	129	531
	June	428	492	412	1 332
	July	29	124	50	203
Six Row C.W.2	April	219	520	127	866
	May	127	218	229	574
	June	221	262	242	725
	July	71	184	82	337
Six Row C.W.3	April	328	500	149	977
	May	361	347	336	1 044
	June	480	376	288	1 144
	July	295	301	164	760
Yellow C.W.3	April	81	85	152	318
	May	118	109	79	306
	June	260	202	272	734
	July	81	75	34	190
Feed No.1	April	232	200	154	586
	May	129	139	212	480
	June	104	200	172	476
	July	70	84	40	194
Feed No.2	April	163	134	1 030	1 327
	May	205	305	201	711
	June	169	236	250	655
	July	76	136	134	346
Feed No.3	April	179	191	164	534
	May	229	298	355	882
	June	317	288	565	1 170
	July	114	124	82	320

Table 38

Data for the Analysis of Variance for 1946

	Six Row		Yellow	Feed Grades			Total of	
	C.W.2	C.W.3	C.W.3	No.1	No.2	No.3	Month	Mean
April	866	977	318	586	1 327	534	4 608	768.00
May	574	1 044	306	480	711	882	3 997	666.17
June	725	1 144	734	476	655	1 170	4 904	817.33
July	337	760	190	194	346	320	2 147	357.83
Total of Grade	2 502	3 925	1 548	1 736	3 039	2 906	15 656	
Mean	625.50	981.25	387.0	434.0	759.75	726.5		

Necessary Difference for Grades 105.93

Necessary Difference for Months 86.49

Totals of Replications

	I	II	III
April	1 202	1 630	1 776
May	1 169	1 416	1 412
June	1 551	1 564	1 789
July	707	904	536

Table 39

Average Number on Duplicate Plates for 1947

Grade	Months	Replications I	II	III	Total of Month
Two Row C.W.2	May	103	125	80	308
	June	182	178	375	735
	July	50	71	87	208
	August	57	51	31	139
Six Row C.W.2	May	240	225	168	633
	June	145	113	231	489
	July	99	71	50	220
	August	159	289	157	605
Six Row C.W.3	May	585	525	685	1 795
	June	625	299	380	1 304
	July	179	371	255	805
	August	239	286	372	897
Yellow C.W.2	May	530	245	3 125	3 900
Dil. 1:1 000	July	17	94	500	611
Yellow C.W.3	May	35	31	70	136
	June	141	174	212	527
	July	53	146	106	305
	August	32	51	25	108
Feed No.1	May	199	200	192	591
	June	81	179	238	498
	July	142	72	105	319
	August	13	64	7	84
Feed No.2	May	285	695	267	1 247
	June	258	173	455	886
	July	254	205	136	595
	August	21	67	62	150
Feed No.3	May	1 215	1 165	955	3 335
	June	376	291	655	1 322
	July	202	139	279	620
	August	236	236	203	675

Table 40

Data for the Analysis of Variance for 1947

	Six Row		Yellow	Feed Grades			Total of	
	C.W.2	C.W.3	C.W.3	No.1	No.2	No.3	Month	Mean
May	633	1 795	136	591	1 247	3 335	7 737	1 289.5
June	489	1 304	527	498	886	1 322	5 026	837.7
July	220	805	305	319	595	620	2 864	477.3
August	605	897	108	84	150	675	2 519	419.8
Total of Grade	1 947	4 801	1 076	1 492	2 878	5 952	18 146	
Mean	486.75	1 200.2	269	373	719.5	1 488		

Necessary Difference for Grades 78.13

Necessary Difference for Months 63.78

Totals of Replications

	I	II	III
May	2 559	2 841	2 337
June	1 626	1 229	2 171
July	929	1 004	931
August	700	993	826

Fungi

Table 41

Average Number on Duplicate Plates for 1946

(Counts in Dilution 1:1 000)

Grade	Months	Replications			Total of Month
		I	II	III	
Two Row C.W.1 Dil. 1:100	April	95	11	26	132
Two Row C.W.2 Dil. 1:100	May	15	11	14	40
	June	10	10	12	32
	July	41	23	25	89
Six Row C.W.2	April	15	9	10	34
	May	6	5	7	18
	June	5	5	2	12
	July	4	1	3	8
Six Row C.W.3	April	18	19	17	54
	May	22	8	8	38
	June	9	8	7	24
	July	8	11	8	27
Yellow C.W.3	April	15	20	71	106
	May	7	6	8	21
	June	17	9	4	30
	July	30	2	5	37
Feed No.1	April	19	17	8	44
	May	8	9	14	31
	June	9	12	9	30
	July	6	11	6	23
Feed No.2	April	12	12	12	36
	May	6	6	10	22
	June	10	10	7	27
	July	12	7	11	30
Feed No.3	April	24	12	23	59
	May	4	5	9	18
	June	19	7	33	59
	July	103	13	10	126

Table 42

Data for the Analysis of Variance for 1946

	Six Row		Yellow	Feed Grades			Total of	
	C.W.2	C.W.3	C.W.3	No.1	No.2	No.3	Months	Mean
April	34	54	106	44	36	59	333	55.50
May	18	38	21	31	22	18	148	24.67
June	12	24	30	30	27	59	182	30.33
July	8	27	37	23	30	126	251	41.83
Total of Grade	72	143	194	128	115	262	914	
Mean	18	35.75	48.5	32	28.75	65.5		

Totals of Replications

	I	II	III
April	103	89	141
May	53	39	56
June	69	51	62
July	163	45	43

Table 43

Average Number on Duplicate Plates for 1947

(Counts in Dilution 1:100)

Grade	Months	Replications I	Replications II	Replications III	Total of Month
Two Row C.W.2	May	12	15	22	49
	June	21	22	28	71
	July	22	41	34	97
	August	14	20	18	52
Six Row C.W.2	May	90	115	50	255
	June	17	17	22	56
	July	15	19	33	67
	August	155	365	110	630
Six Row C.W.3	May	110	95	40	245
	June	47	26	17	90
	July	27	23	25	75
	August	235	310	305	850
Yellow C.W.2	May	6	17	9	32
	July	76	15	22	113
Yellow C.W.3	May	14	14	17	45
	June	46	36	30	112
	July	12	13	15	40
	August	31	31	35	97
Feed No.1	May	75	85	75	235
	June	37	20	41	98
	July	33	47	34	114
	August	12	25	16	53
Feed No.2	May	105	90	70	265
	June	50	35	2 050	2 135
	July	85	230	54	369
	August	20	21	21	62
Feed No.3	May	70	185	140	395
	June	30	31	100	161
	July	31	35	36	102
	August	255	200	295	750

Table 44

Data for the Analysis of Variance for 1947

	Six Row		Yellow	Feed Grades			Total of	
	C.W.2	C.W.3	C.W.3	No.1	No.2	No.3	Month	Mean
May	255	245	45	235	265	395	1 440	240.00
June	56	90	112	98	2 135	161	2 652	442.00
July	67	75	40	114	369	102	767	127.80
August	630	850	97	53	62	750	2 442	407.00
Total of Grade	1 008	1 260	294	500	2 831	1 408	7 301	
Mean	252	315	73.5	125	707.8	352		

Totals of Replications

	I	II	III
May	464	594	392
June	227	165	2 260
July	203	367	197
August	708	952	782

Yeasts

Table 45

Average Number on Duplicates Plates for 1946

(Counts in the Dilution 1:10 000)

Grade	Months	Replications I	II	III	Total of Month
Two Row C.W.1 Dil. 1:100	April	180	18	29	227
Two Row C.W.2 Dil. 1:1 000	May	162	115	156	433
	June	44	39	22	105
	July	41	300	24	365
Six Row C.W.2	April	33	23	12	68
	May	35	22	25	82
	June	7	3	7	17
	July	5	13	8	26
Six Row C.W.3	April	58	13	58	129
	May	44	33	85	162
	June	14	22	3	39
	July	8	7	7	22
Yellow C.W.3	April	24	29	11	64
	May	13	54	31	98
	June	23	9	9	41
	July	3	4	8	15
Feed No.1	April	45	195	18	258
	May	29	32	28	89
	June	11	11	17	39
	July	6	24	14	44
Feed No.2	April	40	18	10	68
	May	32	22	50	104
	June	41	6	7	54
	July	12	15	12	39
Feed No.3	April	28	13	13	54
	May	15	14	59	88
	June	7	31	48	86
	July	9	11	29	49

Table 46

Data for the Analysis of Variance for 1946

	Six Row Yellow			Feed Grades			Total of	
	C.W.2	C.W.3	C.W.3	No.1	No.2	No.3	Month	Mean
April	68	129	64	258	68	54	641	106.85
May	82	162	98	89	104	88	623	103.83
June	17	39	41	39	54	86	276	46.00
July	26	22	15	44	39	49	196	32.50
Total of Grade	193	352	218	430	265	277	1 735	
Mean	48.25	88	54.5	107.5	66.25	69.25		

Necessary Difference for Months 15.94

Totals of Replications

	I	II	III
April	228	291	122
May	168	177	278
June	103	82	91
July	43	74	78

Table 47

Average Number on Duplicate Plates for 1947

(Counts in the Dilution 1:1 000)

Grade	Months	Replications			Total of Month
		I	II	III	
Two Row C.W.2	May	7	137	82	226
	June	20	48	52	120
	July	50	36	129	215
	August	180	190	105	475
Six Row C.W.2	May	4	70	29	103
	June	39	65	86	190
	July	73	120	147	340
	August	370	215	150	735
Six Row C.W.3	May	4	109	115	228
	June	66	85	179	330
	July	102	126	150	378
	August	190	230	85	505
Yellow C.W.2	May	4	141	1 370	1 515
	July	3	6	5	14
Yellow C.W.3	May	14	183	58	255
	June	105	161	330	596
	July	45	71	88	204
	August	475	1 120	1 180	2 775
Feed No.1	May	14	133	199	346
	June	32	166	198	396
	July	445	235	380	1 060
	August	250	195	235	680
Feed No.2	May	3	274	290	567
	June	74	67	1 475	1 616
	July	175	195	280	650
	August	230	505	320	1 055
Feed No.3	May	516	170	146	832
	June	46	117	740	903
	July	235	370	280	885
	August	330	445	785	1 560

Table 48

Data for the Analysis of Variance for 1947

	Six Row		Yellow	Feed Grades			Total of	
	C.W.2	C.W.3	C.W.3	No.1	No.2	No.3	Month	Mean
May	103	228	255	346	567	832	2 331	388.50
June	190	330	596	396	1 616	903	4 031	671.85
July	340	378	204	1 060	650	885	3 517	586.16
August	735	505	2 775	680	1 055	1 560	7 310	1 218.33
Total of Grade	1 368	1 441	3 830	2 482	3 888	4 180	17 189	
Mean	342	360.25	957.5	620.5	972	1 045		

Necessary Difference for Grades 182.63

Necessary Difference for Months 149.10

Total of Replications

	I	II	III
May	555	939	837
June	362	661	3 008
July	1 075	1 117	1 325
August	1 845	2 710	2 755

OATS

Bacteria

(Counts in the Dilution 1:100 000)

Table 49

Average Number on Duplicate Plates før 1946

Grade	Months	Replications I	II	III	Total of Month
C.W.1	April	141	195	172	508
Dil. 1:1 000	June	3 250	1 060	815	5 125
C.W.2	April	52	41	85	178
	May	52	154	18	284
	June	8	11	4	23
	July	37	27	163	227
C.W.3	April	82	71	70	223
	May	79	115	82	276
	June	33	15	9	57
	July	37	34	21	92
C.W.3 extra	April	81	81	26	188
	May	64	53	43	160
	June	19	15	8	42
	July	8	25	6	39
Feed No.1 extra	April	97	66	81	244
	May	65	140	105	310
	July	39	39	17	95
Feed No.1	April	94	45	63	202
	May	69	114	56	239
	June	103	31	16	150
	July	14	25	19	58
Feed No.2	April	246	88	80	414
	May	173	177	202	552
	June	39	63	65	167
	July	58	59	42	159
Feed No.3	April	252	190	150	592
	May	158	125	164	447
	June	36	28	21	85
	July	30	61	47	138

Table 50

Data for the Analysis of Variance for 1946

	C.W.2	C.W.3	C.W.3 extra	Feed Grades No.1	No.2	No.3	Total of Month	Mean
April	178	223	188	202	414	592	1 797	299.50
May	284	276	160	239	552	447	1 958	326.33
June	23	57	42	150	167	85	524	87.33
July	227	92	39	58	159	138	713	118.83
Total of Grade	712	648	429	649	1 292	1 262	4 992	
Mean	178	162	107.25	162.25	323	315.5		

Necessary Difference for Grades 27.74

Necessary Difference for Months 22.65

Totals of Replications

	I	II	III
April	807	516	474
May	595	738	625
June	238	163	123
July	184	231	298

Table 51

Average Number on Duplicate Plates for 1947

Grade	Months	Replications I	II	III	Total of Month
C.W.2	May	29	34	35	98
	June	19	82	66	167
	July	8	8	11	27
	August	21	14	28	63
C.W.3	May	79	32	64	175
	June	19	30	63	112
	July	27	19	7	53
	August	133	105	90	328
C.W.3 extra	May	45	44	38	127
	June	22	19	47	88
	July	4	4	10	18
	August	8	7	8	23
Feed No.1 extra	May	168	82	186	436
	June	63	45	48	156
	July	48	24	18	90
Feed No.1	May	86	136	50	272
	June	61	26	49	136
	July	24	10	20	54
	August	72	82	121	275
Feed No.2	May	316	140	47	503
	June	96	85	26	207
	July	27	70	47	144
	August	178	175	295	648
Feed No.3	May	42	318	48	408
	June	80	128	39	247
	July	104	20	110	234
	August	272	361	448	1 081

Table 52

Data for the Analysis of Variance for 1947

	C.W.2	C.W.3	C.W.3 extra	Feed Grades No.1	No.2	No.3	Total of month	Mean
May	98	175	127	272	503	408	1 583	263.83
June	167	112	88	136	207	247	957	159.50
July	27	53	18	54	144	234	530	88.33
August	63	328	23	275	648	1 081	2 418	403.00
Total of Grade	355	668	256	737	1 502	1 970	5 488	
Mean	88.75	167	64	184.25	375.5	492.5		

Necessary Difference for Grades 43.78

Necessary Difference for Months 35.74

Totals of Replications

	I	II	III
May	597	704	282
June	260	370	290
July	194	131	205
August	684	744	990

Fungi

Table 53

(Counts in the Dilution 1:1 000)

Average Number on Duplicate Plates for 1946

Grade	Months	Replications I	Replications II	Replications III	Total of Month
C.W.1 Dil. 1:100	April	13	22	6	41
	June	47	45	18	110
C.W.2	April	14	10	11	35
	May	40	18	14	72
	June	2	2	4	8
	July	3	5	4	12
C.W.3	April	26	13	13	52
	May	18	16	25	59
	June	4	30	29	63
	July	5	10	5	20
C.W.3 extra	April	10	14	11	35
	May	11	13	13	37
	June	1	12	2	15
	July	9	7	3	19
Feed No.1 extra	April	12	11	26	49
	May	20	7	15	42
	July	21	8	10	39
Feed No.1	April	15	23	9	47
	May	21	15	12	48
	June	11	14	9	34
	July	17	7	9	33
Feed No.2	April	38	20	140	198
	May	30	22	31	83
	June	36	25	38	99
	July	11	69	14	94
Feed No.3	April	86	75	135	296
	May	21	18	19	58
	June	10	10	9	29
	July	12	1	1	14

Table 54

Data for the Analysis of Variance for 1946

	C.W.2	C.W.3	C.W.3 extra	Feed Grades No.1	No.2	No.3	Total of Month	Mean
April	35	52	35	47	198	296	663	110.50
May	72	59	37	48	83	58	357	59.50
June	8	63	15	34	99	29	248	41.33
July	12	20	19	33	94	14	192	32.00
Total of Grade	127	194	106	162	474	397	1 460	
Mean	31.75	48.5	26.5	40.5	118.5	99.25		

Necessary Difference for Grades 14.05

Necessary Difference for Months 11.52

Totals of Replications

	I	II	III
April	189	155	319
May	141	102	114
June	64	93	91
July	57	99	36

Table 55

Average Number on Duplicate Plates for 1947

Grade	Months	Replications			Total of Month
		I	II	III	
C.W.2	May	215	105	15	335
	June	25	23	50	98
	July	30	35	40	105
	August	30	11	19	60
C.W.3	May	125	105	150	380
	June	30	20	32	82
	July	60	80	145	285
	August	205	275	285	765
C.W.3 extra	May	140	95	25	260
	June	16	32	23	71
	July	9	13	5	27
	August	88	30	34	152
Feed No.1 extra	May	45	200	115	360
	June	13	13	18	44
	July	15	100	135	250
Feed No.1	May	65	85	40	190
	June	49	34	49	132
	July	65	120	260	445
	August	160	28	22	210
Feed No.2	May	95	175	150	420
	June	29	30	310	369
	July	36	25	32	93
	August	390	900	900	2 190
Feed No.3	May	10	500	165	675
	June	60	41	35	136
	July	50	37	56	143
	August	1 450	1 700	600	3 750

Table 56

Data for the Analysis of Variance for 1947

	C.W.2	C.W.3	C.W.3 extra	Feed Grades No.1	No.2	No.3	Total of Month	Mean
May	335	380	260	190	420	675	2 260	376.67
June	98	82	71	132	369	136	888	148.00
July	105	285	27	445	93	143	1 098	183.00
August	60	765	152	210	2 190	3 750	7 127	1 187.83
Total of Grade	598	1 512	510	977	3 072	4 704	11 373	
Mean	149.5	378	127.5	244.25	768	1 176		

Necessary Difference for Grades 123.77

Necessary Difference for Months 101.06

Totals of Replications

	I	II	III
May	650	1 065	545
June	209	180	499
July	250	310	538
August	2 323	2 944	1 860

Yeasts

(Counts in the Dilution 1:1 000)

Table 57

Average Number on Duplicate Plates for 1946

Grade	Months	Replicates I	Replicates II	Replicates III	Total of Month
C.W.1	April	10	62	21	93
	June	64	54	21	140
C.W.2	April	132	235	240	607
	May	50	1 000	335	1 385
	June	17	26	9	52
	July	27	10	60	97
C.W.3	April	242	225	80	547
	May	210	470	315	995
	June	95	44	14	153
	July	69	99	116	284
C.W.3 extra	April	236	210	400	846
	May	287	480	115	882
	June	49	55	42	146
	July	38	129	36	203
Feed No.1 extra	April	107	165	410	682
	May	280	200	345	825
	July	24	48	59	131
Feed No.1	April	85	240	210	535
	May	345	325	570	1 240
	June	110	600	29	739
	July	23	71	44	138
Feed No.2	April	363	700	170	1 233
	May	520	125	75	720
	June	165	155	9	329
	July	63	41	53	157
Feed No.3	April	275	675	965	1 915
	May	405	75	410	890
	June	120	140	43	303
	July	79	14	7	100

Table 58

Data for the Analysis of Variance for 1946

	C.W.2	C.W.3	C.W.3 extra	Feed Grades No.1	No.2	No.3	Total of Month	Mean
April	607	547	846	535	1 233	1 915	5 683	947.17
May	1 385	995	882	1 240	720	890	6 112	1 018.67
June	52	153	146	739	329	303	1 722	287.00
July	97	284	203	138	157	101	979	163.17
Total of Grade	2 141	1 979	2 077	2 652	2 439	3 208	14 496	
Mean	535.25	494.8	519.25	663	609.8	802		

Necessary Difference for Months 117.46

Totals of Replications

	I	II	III
April	1 333	2 285	2 065
May	1 817	2 475	1 820
June	556	1 020	146
July	299	364	316

Table 59

Average Number on Duplicate Plates for 1947

Grade	Months	Replications I	II	III	Total of Month
C.W.2	May	14	90	20	124
	June	26	45	39	110
	July	19	57	16	92
	August	22	21	185	228
C.W.3	May	117	155	78	350
	June	53	68	159	280
	July	59	82	51	192
	August	65	70	36	171
C.W.3 extra	May	17	65	22	104
	June	81	57	60	198
	July	42	26	43	111
	August	67	108	44	219
Feed No.1 extra	May	69	100	131	300
	June	44	25	17	86
	July	67	108	44	219
Feed No.1	May	138	160	56	354
	June	515	42	68	625
	July	102	85	50	237
	August	49	15	35	99
Feed No.2	May	173	260	144	577
	June	330	68	99	497
	July	146	37	38	221
	August	110	110	50	270
Feed No.3	May	1	140	140	281
	June	105	52	90	247
	July	79	9	69	157
	August	640	440	295	1 375

Table 60

Data for the Analysis of Variance for 1947

	C.W.2	C.W.3	C.W.3 extra	Feed Grades No.1	No.2	No.3	Total of Month	Mean
May	124	350	104	354	577	281	1 790	298.33
June	110	280	198	625	497	247	1 957	326.17
July	92	192	111	237	221	157	1 010	168.33
August	228	171	219	99	270	1 357	2 362	393.67
Total of Grade	554	993	632	1 315	1 565	2 060	7 119	
Mean	138.5	248.25	158	328.8	391.25	515		

Necessary Difference for Grades 68.94

Totals of Replications

	I	II	III
May	460	870	460
June	1 110	332	515
July	447	296	267
August	953	764	645

HULLESS OATS

Table 61

Average Number of Bacteria, Fungi, Yeasts and Sporeformers on Duplicate Plates for 1946 and 1947

Organisms	Dilution	Months	1946 Replications			1947 Replications		
			1	2	3	1	2	3
Bacteria	1:1 000	I	161	89	100	82	8	150
		II	1 045	450	475	32	19	35
		III	50	115	65	4	6	16
		IV	73	145	41	130	16	60
Fungi	1:100	I	75	50	47	520	9	12
		II	69	80	85	2	4	0
		III	70	35	32	3	5	5
		IV	29	22	44	6	1	1
Yeasts	1:100	I	19	25	23	640	20	20
		II	40	280	25	10	1	8
		III	670	485	3	2	0	1
		IV	17	17	14	12	1	7
Sporeformers	1:100	I	not determined			14	2	-
		II				3	1	13
		III				1	2	1
		IV				2	1	1

BACTERIAL SPORES

(Counts in the Dilution 1:100)

FLAX

Table 62

Average Number on Duplicate Plates for 1947

Grade	Months	Replications I	II	III	Total of Month
C.W.1	May	6	7	22	35
	June	3	9	6	18
	July	10	7	5	22
C.W.2	May	20	13	9	42
	June	2	2	6	10
	July	11	10	28	49
	August	5	8	3	16
C.W.3	May	20	10	12	42
	June	8	5	7	20
	July	15	7	3	25
	August	18	10	4	32
C.W.4	June	18	28	28	74
	July	13	4	15	32

Table 63

Data for the Analysis of Variance

	C.W.1	C.W.2	C.W.3	Total of Month	Mean
May	35	44	43	122	40.67
June	18	11	20	49	16.33
July	22	49	25	96	32.00
August	19	16	32	67	22.33
Total of Grade	94	120	120	334	
Mean	23.5	30	30		

	Totals of Replications		
	I	II	III
May	48	31	43
June	13	16	20
July	36	24	36
August	24	28	15

Table 64

Analysis of Variance

Source of Variation	Mean Square	D.F.	F.	5% Pt.
Grades	18.77	2	0.52	3.40
Months	114.85	3	3.21	3.01
M x G	31.30	6	0.87	2.51
Error	35.80	24	-	-

BARLEY

Table 65

Average Number on Duplicate Plates for 1947

Grade	Months	Replications I	II	III	Total of Month
Two Row C.W.2	May	1	1	7	9
	June	1	3	4	8
	July	2	1	2	5
	August	12	9	7	28
Six Row C.W.2	May	2	3	10	15
	June	5	1	5	11
	July	3	1	4	8
	August	6	1	3	10
Six Row C.W.3	May	1	0	4	5
	June	2	1	9	12
	July	3	3	2	8
	August	17	8	6	31
Yellow C.W.2	May	7	7	9	23
	July	6	1	7	14
Yellow C.W.3	May	3	1	3	7
	June	4	19	16	39
	July	8	2	2	12
	August	98	6	7	111
Feed No.1	May	12	3	5	20
	June	2	2	9	13
	July	7	4	5	16
	August	45	28	35	108
Feed No.2	May	5	2	8	15
	June	6	2	21	26
	July	6	10	8	24
	August	14	13	14	41
Feed No.3	May	6	6	14	26
	June	1	52	15	68
	July	6	4	7	17
	August	2	6	14	22

Table 66

Data for the Analysis of Variance

	Six Row		Yellow	Feed Grades			Total of	
	C.W.2	C.W.3	C.W.3	No.1	No.2	No.3	month	mean
May	15	5	7	20	15	23	88	14.67
June	11	12	39	13	26	68	169	28.17
July	8	8	12	16	25	17	85	14.17
August	10	31	111	108	41	22	323	53.83
Total of Grade	44	56	169	157	106	133	665	
Mean	11	14	42.25	39.25	26.5	33.33		

Totals of Replications

	I	II	III
May	29	15	44
June	17	77	75
July	33	24	28
August	182	62	79

Table 67

Analysis of Variance

Source of Variation	Mean Square	D.F.	F.	5% Pt.
Grades	225.05	5	1.37	2.41
Months	690.79	3	4.20	2.80
G x M	210.58	15	1.28	1.88
Error	164.56	48		

OATS

Table 68

Average Number on Duplicate Plates for 1947

Grade	Months	Replications I	II	III	Total of Month
C.W.2	May	1	3	-	4
	June	2	1	3	6
	July	1	6	4	11
	August	3	7	3	13
C.W.3	May	1	7	-	8
	June	2	5	2	9
	July	5	6	2	13
	August	9	12	-	21
C.W.3 extra	May	3	9	-	12
	June	9	3	2	14
	July	1	2	8	11
	August	3	5	3	11
Feed No.1 extra	May	1	1	-	2
	June	4	2	1	7
	July	30	1	3	34
Feed No.1	May	12	397	-	409
	June	800	5	34	839
	July	10	6	9	25
	August	7	44	375	426
Feed No.2	May	7	6	-	13
	June	13	8	49	70
	July	t.n.	17	17	t.n.
	August	82	36	30	148
Feed No.3	May	14	t.n.	-	t.n.
	June	8	14	28	50
	July	8	27	47	82
	August	19	33	39	91

t.n. = colonies too numerous to count

Table 69

Total Counts and Numbers of Surface Colonies for 1946

Crop	Grade	Dilution	Total Count	Surface Colonies
FLAX	C.W.1	1:100 000	3 057	508
	C.W.2		21 414	3 606
	C.W.3		17 085	2 688
	C.W.4		734	147
	Total		42 290	6 949
OATS	C.W.1	1:10 000	440	47
	C.W.2		7 474	1 447
	C.W. extra 3		4 606	800
	C.W.3		4 632	673
	Feed No.1		10 105	1 576
	No.2		13 262	1 909
	No.3		13 375	1 957
	Total		53 894	8 409

Table 69 continued

Total Counts and Numbers of Surface Colonies for 1947

Crop	Grade	Dilution	Total Count	Surface Colonies
FLAX	C.W.1	1 : 10 000	48 970	9 777
	C.W.2		124 810	17 380
	C.W.3		85 637	16 487
	C.W.4		40 950	6 790
	Total		300 367	50 434
BARLEY	Two Row C.W.2	1 : 100 000	4 226	368
	Six Row C.W.2		3 900	359
	C.W.3		9 597	647
	Yellow C.W.2		1 162 300	110 300
	C.W.3		2 157	257
	Feed Grade No.1		2 970	282
	No.2		5 761	581
	No.3		11 908	596
	Total		1 202 819	113 390
OATS	C.W.2	1 : 100 00	7 188	1 050
	C.W. extra 3		5 122	784
	C.W.3		13 089	1 365
	extra Feed No.1	1 : 100 000	1 370	177
	Feed Grade No.1		1 483	184
	No.2		2 955	327
	No.3		3 947	340
	Total		35 154	4 227

Table 70

Change in pH of 38 Cultures after 5 Days Incubation at 26 degrees C.

(in relation to uninoculated checks)

Culture No.	Source	Glucose	Fructose	Galactose
1	Wheat	-2.80	-1.05	-1.95
2		-3.05	-1.25	-2.55
3		-2.95	-1.05	-2.55
4		-2.70	-1.00	-2.25
5		-2.70	-1.20	-2.40
6		-2.85	-1.20	-2.05
7		-2.60	-1.05	-2.40
8		-4.75	-1.20	-2.40
9		-4.75	-1.00	-2.40
10	Barley	-2.55	-0.90	-2.05
11		-2.90	-0.95	-2.20
12		-2.80	-1.10	-2.60
13		-2.80	-0.95	-2.50
14		-2.60	-0.95	-2.30
15		-2.60	-1.30	-2.30
16		-2.85	-1.30	-2.45
17		-3.00	-0.90	-2.50
18		-2.75	-0.90	-2.40
19		-3.05	-1.10	-2.50
20	Oats	-2.80	-1.25	-1.90
21		-3.05	-1.35	-2.60
22		-2.80	-0.85	-2.15
23		-2.70	-0.95	-1.90
24		-2.75	-1.25	-2.25
25		-2.75	-0.95	-2.40
26		-3.10	-1.35	-2.40
27		-2.85	-0.85	-2.10
28		-2.70	-1.10	-2.00
29	Flax	-2.90	-0.95	-2.25
30		-2.95	-1.15	-2.25
31		-2.80	-1.15	-2.55
32		-2.95	-1.10	-2.10
33		-2.80	-0.90	-2.50
34		-2.90	-1.15	-2.50
35		-2.80	-1.15	-2.25
36		-2.80	-1.25	-2.30
37		-2.75	-1.05	-2.20
38		-2.75	-1.05	-2.30

Table 70 continued

Culture No.	Source	Mannose	Lactose	Sucrose	Maltose
1	Wheat	-2.80	-0.10	-1.30	-0.30
2		-2.85	-0.35	-1.20	-0.50
3		-2.80	-0.25	-0.90	-0.50
4		-2.80	-0.30	-0.25	-0.35
5		-2.70	-0.30	-0.35	-0.25
6		-2.90	-0.30	-1.20	-0.55
7		-2.70	-0.45	-2.05	-0.80
8		-2.70	-0.40	-2.40	-0.30
9		-2.60	-0.40	-0.45	-0.30
10	Barley	-2.85	-0.25	-0.70	-1.15
11		-2.70	-0.25	-2.00	-0.45
12		-2.80	-0.30	-2.40	-0.90
13		-2.55	-0.35	-0.40	-0.25
14		-2.85	-0.30	-1.45	-0.30
15		-2.55	-0.40	-0.60	-0.15
16		-2.80	-0.40	-1.35	-0.20
17		-2.80	-0.30	-0.75	-0.25
18		-2.80	-0.45	-0.50	-0.25
19		-2.75	-0.45	-1.90	-0.25
20	Oats	-2.60	-0.15	-0.60	-0.35
21		-2.90	-0.80	-1.80	-0.40
22		-2.70	-0.15	-1.50	-0.45
23		-2.65	-0.15	-1.15	-0.60
24		-2.65	-0.30	-1.25	-0.20
25		-2.55	-0.40	-0.55	-0.10
26		-2.75	-0.40	-0.20	-0.25
27		-2.70	-0.50	-1.75	-0.30
28		-2.55	-0.40	-0.40	-0.30
29	Flax	-2.80	-0.30	-0.90	-0.35
30		-2.70	-0.15	-0.90	-0.35
31		-2.75	-0.30	-0.60	-0.65
32		-2.75	-0.20	-0.70	-0.35
33		-2.75	-0.30	-0.80	-0.35
34		-2.70	-0.35	-1.40	-0.35
35		-2.70	-0.40	-1.60	-0.20
36		-2.70	-0.35	-1.95	-0.40
37		-2.75	-0.30	-0.70	-0.20
38		-2.75	-0.35	-2.00	-0.20

Table 70 continued

Culture No.	Source	Raffinose	Xylose	Arabinose	Rhamnose
1	Wheat	+0.05	-3.30	-2.70	-1.60
2		+0.25	-3.40	-1.50	-1.35
3		0.00	-3.35	-3.00	-1.75
4		+0.10	-3.60	-3.00	-1.90
5		-0.05	-3.40	-2.50	-1.15
6		+0.35	-3.15	-1.35	-1.10
7		0.00	-3.50	-1.40	-1.45
8		+0.35	-3.55	-2.60	-1.45
9		+0.25	-3.45	-1.50	-1.15
10	Barley	-0.10	-3.35	-2.65	-1.25
11		-0.20	-2.50	-2.80	-0.95
12		-0.10	-3.30	-2.55	-0.85
13		+0.10	-3.25	-2.80	-1.20
14		-0.05	-2.40	-2.55	-1.60
15		-0.05	-3.30	-3.00	-1.20
16		-0.05	-3.35	-3.05	-1.45
17		-0.05	-3.05	-3.10	-1.15
18		-0.05	-3.30	-3.30	-0.85
19		-0.05	-3.50	-3.10	-1.30
20	Oats	-0.15	-2.80	-1.60	-1.40
21		-1.25	-2.90	-3.55	-1.90
22		0.00	-3.00	-3.20	-1.95
23		0.00	-2.20	-3.20	-1.95
24		-0.05	-3.10	-3.35	-2.15
25		-0.05	-0.90	-3.30	-1.40
26		0.00	-2.90	-1.00	-3.25
27		0.00	-3.20	-3.05	-1.75
28		-0.10	-2.55	-3.20	-1.70
29	Flax	-0.10	-1.20	-2.55	-1.10
30		-0.20	-4.00	-3.25	-0.45
31		+0.10	-3.40	-3.30	-1.30
32		-0.20	-3.25	-1.70	-1.30
33		0.00	-3.00	-3.40	-1.85
34		0.00	-3.20	-3.35	-1.20
35		0.00	-3.05	-3.20	-1.25
36		0.00	-3.30	-3.35	-1.00
37		0.00	-2.65	-3.15	-0.20
38		0.00	-3.50	-2.75	-0.65

Table 70 continued

Culture No.	Source	Starch	Dextrin	Inulin	Salicin
1	Wheat	+0.25	+1.10	+1.05	-1.10
2		+0.15	+1.10	+1.25	
3		+0.15	+1.05	+1.25	
4		+0.20	+0.85	+1.30	
5		0.00	+1.35	+1.35	
6		+0.30	+1.05	+1.10	
7		0.00	+0.90	+1.25	
8		+0.30	+0.75	+1.30	
9		+0.15	+0.80	+1.20	
10	Barley	+0.25	+0.40	+1.10	-1.30
11		+0.30	+0.45	+1.45	
12		+0.25	+0.45	+1.50	
13		+0.40	+0.25	+1.15	
14		+0.20	+0.05	+0.80	
15		+0.30	-0.25	+1.35	
16		+0.20	+0.05	+1.45	
17		+0.35	+0.05	+1.20	
18		+0.35	+0.05	+1.35	-1.20
19		-0.40	0.00	+1.60	
20	Oats	+0.05	+0.85	+1.60	
21		-0.10	+0.10	+0.85	-0.70
22		+0.10	+0.10	+1.10	
23		+0.20	+0.35	+0.95	
24		+0.15	+0.30	+0.75	
25		+0.10	-0.05	+1.35	
26		0.00	+0.20	+1.65	-2.00
27		+0.20	+0.15	+0.95	
28		-0.10	-0.05	+1.20	
29	Flax	+0.85	+0.75	+1.35	
30		+1.05	+0.45	+1.20	-0.80
31		+1.60	+0.65	+1.35	
32		+0.90	+0.85	+1.45	
33		+0.95	+1.60	+1.30	
34		+0.85	+0.80	+0.95	
35		+0.40	+0.90	+1.50	
36		+0.90	+0.85	+1.30	
37		+0.60	+0.85	+1.50	-1.00
38		+0.70	+0.95	+1.50	

Table 71

Change in pH of 52 Cultures after 5 Days Incubation at 26 degrees C.

(in relation to uninoculated checks)

Culture No.	Source	Glucose	Lactose	Sucrose	Salicin
1	Wheat	-2.95	-0.05	+0.25	0.00
2		-1.05	-0.05	-1.55	0.00
3		-1.95	-0.15	-0.15	0.00
4		-2.95	-0.15	-0.25	0.00
5		-3.05	-0.05	-0.45	0.00
6	Flax	-0.65	-0.05	-0.30	0.00
7		-0.55	-0.05	-0.40	+0.20
8		-3.35	+0.05	+0.10	0.00
9		-1.85	-0.05	+0.40	-0.10
10		-0.65	+0.05	-1.30	0.00
11		-1.65	-0.05	-0.20	+0.20
12		-2.15	+0.05	0.00	+0.10
13		-2.05	-0.05	0.00	-0.10
14		-2.55	-0.05	-1.10	+0.20
15		-3.15	-0.15	-2.50	+0.10
16	Barley	-2.05	-0.35	+0.10	0.00
17		-0.95	+0.05	0.00	+0.10
18		-2.15	+0.35	-2.00	0.00
19		-2.45	+0.05	-2.20	0.00
20		-1.65	+0.55	-0.70	+0.10
21		-2.45	-0.05	0.00	+0.10
22		-1.95	+0.25	-0.50	+0.10
23		-1.65	+0.25	-0.80	0.00
24		-1.95	+0.35	-0.80	-0.10
25		-1.55	+0.15	-1.90	-0.10
26		-3.05	-0.15	-0.25	-0.10
27		-2.75	-0.35	-0.25	-0.10
28	Corn	-0.85	-0.05	+0.05	-0.10
29		-2.15	-0.05	-0.35	-0.10
30		-1.05	-0.25	-0.05	-0.10
31	Oats	-2.85	-0.25	+0.35	0.00
32		-2.95	-0.25	+0.25	0.00
33		+0.45	+0.35	+0.15	0.00
34		-2.55	+0.25	+0.15	0.00
35		-2.75	+0.05	+0.25	0.00
36		-2.65	+0.05	-0.05	0.00
37		-0.65	-0.05	-0.05	0.00

Table 71 continued

Culture No.	Source	Glucose	Lactose	Sucrose	Salicin
38	Oats	-1.85	+0.15	-2.45	0.00
39		-2.05	+0.05	-0.35	+0.10
40		-0.60	+0.45	+0.15	-0.10
41		-0.75	-0.25	-1.15	0.00
42		-1.10	-0.15	+0.15	0.00
43		-2.65	-0.25	+0.35	-0.20
44		-2.20	+0.05	-0.05	0.00
45		-1.90	+0.25	-0.25	0.00
46		-2.75	+0.15	-0.95	-0.10
47		-2.85	-0.35	+0.05	-0.10
48		-1.20	+0.25	-1.65	0.00
49		-1.65	-0.05	-2.45	0.00
50		-0.75	-0.25	-0.25	0.00
51		-2.45	-0.05	-2.55	0.00
52		-0.60	+0.25	-0.35	0.00