INCIDENCE OF YELLOW CHROMOGENIC BACTERIA ON CERTAIN PLANTS

By

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INTRODUCTION

For many years, investigators have known that leaves, stems and seeds of plants harbour bacteria, yeasts, and fungi. Duggeli (8) found large numbers of bacteria on the surfaces of healthy plants, the most common form being a yellow chromogen, which he named Bacterium herbicola aureum. He regarded these organisms to be true epiphytes peculiar to the surface of plants. According to Lehmann, Neumann, and Breed (21), this name was changed by a student of Duggeli, named Geilinger, to the binomial Bacterium herbicola. Beijerinck (4) claimed that Duggeli's culture was the same as the one named Bacillus agglomerans earlier by him. Winkler (35) gave the name Bacillus mesentericus aureus to a yellow chromogen isolated from the surface of plum leaves.

Huss (14) isolated from clover hay a yellow-pigmented, polar-flagellated, motile rod which produced agar-slant cultures with an agreeable odor. He named it Pseudomonas trifolii. According to Mack (22), all the above names apply to the same species as does Pseudomonas trifolii.

In many cases, yellow, non-pathogenic bacteria on plate cultures have been mistaken for certain pathogens. E. F. Smith (28) found this to occur frequently in the case of olive knots. This investigator obtained a large number of non-pathogenic, yellow bacteria from the surface of corn kernels, from crown-gall of peach, from daisy gall, and from rose gall, practically to the exclusion of the pathogenic organisms. An experiment station bulletin (28) on the bacterial spot

of carnation was based on the wrong organism, a common surface-growing yellow type. Waite (28) also found a yellow, non-parasitic chromogen associated with Bacterium malvacearum on the cotton plant. Grieg Smith (28) isolated a red chromogen from sugar cane, attacked by Cobb's disease, and ascribed the red strands to it. However, E. F. Smith (28) found only yellow bacteria in the red strands.

Further reports on the presence of yellow-pigmented bacteria on plants include those by Zikes (22) on bran; Kursteiner (22) on straw; Wigger (22) on bran; Chraszcz (22) on hull of barley seeds; Morgenthaler (23) on green malt and stored wheat; Fred et al (9) on green sweet corn; Hummer (13) on barley seeds and rootlets; Woller (36) on plants and seeds; Grapengeter (22) on green plants; Huttig (22) on green plants; Keipper et al (22) on cabbage leaves; Mack (22) on sprouted and unsprouted wheat, and on green plants. Mack considered the yellow chromogen isolated by her to represent a species in the genus Flavobacterium. Bojko (3) used the name Flavobacterium herbicola for the species found on stored rye seeds. Kretovich and Rautenstein (20) later referred to this species, found on stored wheat seeds, as Bacterium herbicola.

A number of investigators have shown that, no matter how vigorous the washing and dry cleaning processes in a mill are, wheat kernels harbour on their surface a number of microorganisms, some of which are epiphytic and are incorporated with the flour. Thus, when flour was allowed to ferment in water, a number of organisms of the colon group appeared, among which were found strains resembling Bacillus levans

Lehmann and Wolffine, which formed yellow cultures on agar, and fermented glucose with acid and gas formation. These have been referred to as the "yellow gas formers" of Holliger, one type considered to be Bacterium coli var luteoliquefaciens Lehmann and Levy. Lehmann, Neumann, and Breed (21) suggested that the "yellow acid formers" might be included in Bacterium herbicola.

Hummer (13) observed that in musty grain fungi prevailed while Bacterium herbicola was present only in small numbers. He concluded therefore that a large number of Bacterium herbicola on seeds of cereals was a sign of the good condition of the seed.

Kent-Jones and Amos (1) made estimates of bacteria on samples of wheat passing through the normal channels of trade in Great Britain. One of the predominating organisms which was isolated was a short rod which appeared to be a member of the genus Flavobacterium. Since a large proportion of the epiphytic microflora of green plants has been found to consist of pigmented, short rods, these investigators believed that organisms found on commercial wheat represented typical plant epiphytes rather than soil or dust contaminants.

O'Gara (24) described a disease of western wheat grass, characterized by the presence of masses of surface bacteria which formed a lemon-yellow ooze over the upper portion of the plant including the seed. The causal organism, Aplanobacter agropyri, was a short, non-motile, non-flagellated, Gram-negative rod; producing viscid, yellow, smooth colonies on nutrient agar and on cooked potato and reducing

litmus milk after about four months. He (25) reported that this disease had many characteristics in common with Rathay's disease of orchard grass, caused by Aplanobacter rathayii. In both cases, a characteristic, viscid, lemon-yellow slime formed on the leaves. The injury to the plant was due to the bacteria penetrating into the interior. The causal organisms gave characteristic lemon-yellow growth on neutral nutrient agar.

Jones et al (18) reported a widely occurring bacterial disease of leaves, leaf sheaths, and glumes of barley characterized by lesions with bacterial exudate. Diseases similar to the one on barley have been found on wheat, spelt, and rye; and the causal organisms, monotrichous rods, were found to belong to the same species. However, the barley blight organism attacked barley only, while the wheat, rye and spelt organism infected wheat, rye, spelt and barley. Later (19), they described the bacterial blight organism of barley as a single-polar-flagellated, motile, short, Gram-negative rod, forming colonies on agar that were wax-yellow tinged with old-gold in color, and soft but not viscid in consistency, and gave it the name Bacterium translucens n. sp. This organism was isolated from the tissues and the exudate of barley. It was also isolated from old barley kernels on which it persisted. In this way the disease was carried from year to year.

Smith et al (29) gave the name Bacterium translucens var undulosum to the organism causing black chaff of wheat. The characteristics of this organism were similar to those of Bacterium translucens. On nutrient agar, it produced pale yellow, smooth, homogeneous colonies with minute

waves in the interior which were distinguishable in oblique light, and on potato agar a very pale yellow slime.

Reddy et al (26) isolated the organism causing bacterial blight of rye. This organism, Bacterium translucens secalis, was identical in morphological and physiological characters with Bacterium translucens and Bacterium translucens var undulosum, but differed from both in pathogenicity. Bacterium translucens secalis infected only rye; Bacterium translucens infected only barley; while Bacterium translucens var undulosum infected wheat, barley, rye, and spelt.

Israilsky and Kazakova (15) isolated from black chaff an organism intermèdiate between Bacterium translucens and Bacterium atrofaciens McCulloch. However, later it was found that this isolate represented merely a variant of the former.

Hagborg (11) proposed that the species causing bacterial black chaff be placed in the genus Phytomonas. He isolated bacteria from diseased specimens of the black chaff type, tested them for pathogenicity and found that, while Phytomonas translucens f. sp. undulosum was common during the years of the investigation, it was not the cause of all the discolorations referred to as "black chaff".

Dowson (7) proposed the generic name Xanthomonas for Gram-negative bacterial plant pathogems. These pathogems, isolated from diseased plants, were described as small, polar-flagellated, motile rods, which formed characteristic, yellow, non-diffusible, pigmented growths on solid media. He transferred the original species, Bacterium translucens to

this new genus, as Xanthomonas translucens. Hagborg (12) emended the description of Xanthomonas translucens to include the five 'formae speciales' which were as follows:- Xanthomonas translucens f. sp. hordei f. sp. nov. which infected barley only and occurred naturally on barley; Xanthomonas translucens f. sp. undulosa (S. J. & R.) which infected wheat, barley, and rye, and occurred naturally on wheat and rye; Xanthomonas translucens f. sp. secalis (R. G. & J.) which infected and occurred naturally on rye; Xanthomonas translucens f. sp. hordei-avenae which infected barley and oats and occurred naturally on barley; Xanthomonas translucens f. sp. cerealis which infected wheat, barley, oats, and rye and occurred naturally on wheat.

Starr (31) reported that most species of Xanthomonas grew in a basal medium of NH_4Cl, glucose and salts, but that some species required in addition methionine, glutamic acid, or nicotinic acid. Starr and Weiss (32) found that Xanthomonas spp. did not grow in a synthetic medium and stated that this characteristic might be valuable in the taxonomy of bacterial plant pathogens. Goldstein (10) reported that the yellow epiphyte of wheat produced slight growth in a medium containing asparagine as the sole source of carbon and nitrogen whereas representatives of the genus Xanthomonas produced no appreciable growth.

James et al (16) studied the microflora of stored wheat and found that the majority of the colonies developing on nutrient agar apparently belonged to one species. This type had an average incidence of 30%, 34%, and 37% on No. 6, No. 1, and No. 4 Manitoba Northern red spring wheats, respectively. It appeared to be the same as the epiphytic

species, reported by Duggeli as Bacterium herbicola aureum.

Wilson (34) studied 22 isolates of yellow bacteria from wheat, which resembled culturally and physiologically Phytomonas translucens var undulosa (Smith, Jones, and Reddy) Hagborg--a plant pathogen. She estimated that this type made up about 85% of all the bacteria on wheat.

Simmonds (27) reported that the epiphytic bacteria on wheat seeds were antibiotic to Helminthosporium sativum P. K. & B., a common root pathogen. His description of the isolations made could be applicable to the epiphyte. However, James et al (17) produced evidence that, while cultures of certain yellow chromogens and of the mixed flora of grain suppressed the growth of the fungus, filtrates of the same bacteria failed to do so. They suggested that this apparent antagonistic action of the epiphytic bacteria probably was due to competition for nutriemts in the medium surrounding the seeds, since a numerically increased epiphytic flora would result from the preliminary incubation of the moistened seeds.

Stark (30) carried out a comprehensive study of the flora of seeds of flax, barley, and oats, representing different grades, and on wheat plants. This investigator reported that yellow chromogenic bacteria constituted a large proportion of the flora on seeds and on growing plants, particularly on older plants.

Burkholder (5) claimed that members of the genus Xanthomonas were different from other bacteria with the probable exception of Pseudomonas trifolii Huss. Even though the yellow saprophytes commonly

found on plants were similar in color to Xanthomonas spp., those studied by him were dissimilar in other characteristics.

Goldstein (10) carried out a comparative study of 13 isolates of the yellow epiphyte from wheat and nine representatives of the genus Xanthomonas. She showed that the two groups of organisms were similar morphologically and in most cultural characteristics, while differences in some of the physiological characteristics were no greater than would be found between species in any genus.

Steel (33) compared 31 isolates of the yellow epiphyte from various sources with seven isolates of certain named species of Xanthomonas and found close agreement in nutritional requirements. He observed certain differences, however. The epiphyte grew in the basal medium of Starr to which was added asparagine as the sole source of carbon and nitrogen. It grew also in a nutrient broth medium with a pH of 4.5. He made tests for pathogenicity on wheat, oats, and barley, using 19 isolates of the epiphyte and four representatives of Xanthomonas translucens. None of the isolates of the epiphyte produced symptoms of the disease whereas the pathogens produced the characteristic lesions of black chaff. This investigator also studied the pigments produced by the epiphyte, and found that they were similar to the pathogens in respect to: lipocyan reaction, extracting solvents, partition test, color tests and chromatographic adsorption. However, he found the pigments to be different when observed on a photoelectric spectrophotometer, but, the difference between an epiphyte and a named pathogen was not greater than the difference between species in the genus Xanthomonas.

From the above, it is evident that the surfaces of seeds and the structures of various plants harbour large numbers of yellow chromogenic bacteria, some of which are pathogenic and others saprophytic. The yellow pathogens have been studied in some detail by various investigators, and classified. This investigation was carried out to obtain additional information on the yellow chromogenic saprophytes on plants. It was divided into four parts:-

Part I----Yellow chromogens on certain grains

Part II---Yellow chromogens on certain grasses and shrubs

Part III---Yellow chromogens on normal and germinated wheat

Part IV---Yellow chromogens on clover hay

PART I

YELLOW CHROMOGENS ON CERTAIN GRAINS

PART I

YELLOW CHROMOGENS ON CERTAIN GRAINS

The plants which were used in this part of the study were grown at The University of Manitoba. They represented: Redman wheat, Thatcher wheat, Exeter oats, Montcalm barley, Dakota flax, and Pagoda soybeans. These were sampled at 0 (i. e. when the plant was about 4 - 6 inches high), 7, 19, 31, 49, 55, 66, 84, and 94 days. Additional samples representing the heads or pods were taken at 49 days and at sampling dates thereafter.

Each plant was cut above the ground, high enough to minimize soil contamination, sufficient clippings being used to insure about one gram dry weight. Each sample was washed in 100-ml. sterile water in a 6-oz. screw-top bottle by shaking 25 times and drained thoroughly, in order to remove dust type organisms. It was diluted in 100-ml. sterile water and shaken on a to-and-fro machine for 30 minutes. Appropriate dilutions were plated in duplicate in Difco nutrient agar. Incubation was at 25°C. for 7 days.

In order to determine the weight, the sample was dried in an oven at 100-104°C. for about 16 hours in the original bottle, and the tare determined. Counts representing the numbers of bacteria and yellow chromogens were recorded and adjusted to the one gram dry-weight basis.

In order to segregate the epiphyte all yellow surface colonies on one plate from each sample were subcultured on nutrient agar slants and later tested for:-

(1) Nitrate reduction

Duplicate nitrate broth cultures, incubated at 25°C. for 48 hours, were tested for nitrites by adding a few drops of sulfanilic acid (8 gms. of sulfanilic acid in 1000-ml. of 5 N acetic acid) and α-naphthylamine reagent (5 gms. of α-naphthylamine dissolved in 1000-ml. of 5 N acetic acid) to 0.5 ml. portions of the culture; and for ammonia by adding 4 or 5 drops of Nessler's reagent to other 0.5 ml. portions of the culture.

A typical epiphyte, in this study checked against a culture isolated by Steel (33) in 1950, reduced nitrate to nitrite but not to ammonia. At each date of testing two tubes of uninoculated medium were tested with the reagents, as an additional check on methods.

(2) Growth in a medium containing DL-asparagine as the sole source of carbon and nitrogen

All isolates, cultured in duplicate, were tested for growth in the nutrient medium of Starr and Weiss (32), after incubation at 25°C. for 5 days. The composition of the medium follows:-

Asparagine	1.0 gm.
K_2HPO_4	1.0 gm.
$MgSO_4$	0.2 gm.
$CaCl_2$	0.1 gm.
NaCl	0.1 gm.
$FeCl_3$	1.0 ml. of a 1% solution
Distilled water	1000 ml.

A typical epiphyte in this case produced slight turbidity in the medium, the culture of Steel (33) being used as a

check again. At each date of testing Pseudomonas aeruginosa and Xanthomonas translucens were tested in this same medium under the same conditions. The former regularly produced turbidity; whereas the latter produced no evidence of growth.

(3) Motility

Hanging drop preparations from nutrient broth cultures incubated at 25°C. for 24 hours were examined for motility. A clean sweeping movement or a darting across the microscopic field was taken to be typical of the epiphyte.

(4) Gram's reaction

The same nutrient broth cultures were next tested by Gram's stain. Small, Gram-negative rods were assumed to represent the epiphyte.

The total count data, expressed as averages of counts from four plates, on each of the grains, are presented in Tables IA and IB. Counts of yellow chromogens on these samples are presented in Tables IIA and IIB; and of the epiphyte in Tables IIIA and IIIB. The same data, expressed as logarithms, are shown graphically in Figs. 1 to 11.

In general, counts were low and fluctuated erratically at the first four samplings. However, starting at 49 days, the total count data, as well as the count of yellow chromogens and of the epiphyte, was higher and remained at a high level for the remainder of the investigation. In most cases, the highest count was obtained at the 94-day sampling.

Yellow chromogens constituted from 0 to 88% of the total bacterial population on the grains used. However, they represented less than 50% in about three-quarters of the samples. The results are shown in Tables IVA and IVB. The epiphyte was the only yellow chromogen on 38% of these samples. It made up a large proportion on most of the others. These results are shown in Tables VA and VB.

Table IA

Total bacteria on wheat and oats during the growing season (x 10^4 /gm.)

1951	Redman Wheat Stems and leaves	Redman Wheat Heads	Thatcher Wheat Stems and leaves	Thatcher Wheat Heads	Oats Stems and leaves	Oats Heads
June 15	8[x]	---	7	---	17	---
June 22	31	---	0.5	---	0.3	---
July 7	42	---	10	---	0.1	---
July 16	0.4	---	0.1	---	2	---
August 3	97	649	395	37	886	14
August 9	1,753	3,077	1,419	254	722	120
August 20	31,888	1,197	18,452	40	1,284	243
September 6	72,414	5,837	47,541	3,299	17,674	19,149
September 17	94,556	30,488	128,964	9,915	28,641	15,714

x average of counts from four plates in each case

--- sample not obtained

Table IB

Total bacteria on barley, flax and soybeans

during the growing season (x 10^4 /gm.)

1951	Barley		Flax		Soybeans	
	Stems and leaves	Heads	Stems and leaves	Heads	Stems and leaves	Pods
June 15	19[x]	---	6	---	85	---
June 22	69	---	0.7	---	46	---
July 7	4	---	0.6	---	28	---
July 16	16	---	0.1	---	454	---
August 3	686	341	---	---	---	---
August 9	6,908	1,627	577	734	283	---
August 20	52,174	7,143	3,964	1,016	914	---
September 6	21,007	4,867	73,214	37,200	15,830	---
September 17	89,963	5,065	144,737	24,193	10,458	2,674

x average of counts from four plates in each case

--- sample not obtained

Table IIA

Yellow chromogens on wheat and oats during the growing season (x 10^4 /gm.)

1951	Redman Wheat Stems and leaves	Redman Wheat Heads	Thatcher Wheat Stems and leaves	Thatcher Wheat Heads	Oats Stems and leaves	Oats Heads
June 15	0[xx]	---	0.4[x]	---	1	---
June 22	12	---	0.2	---	0.1	---
July 7	0	---	0	---	0.01	---
July 16	0	---	0	---	0.8	---
August 3	4	578	8	18	97	8
August 9	192	2,477	215	190	206	72
August 20	20,408	485	9,524	7	350	194
September 6	17,241	1,382	16,393	928	11,480	11,550
September 17	57,307	15,244	64,482	3,777	18,932	10,714

x average of counts from four plates in each case

xx no count on any plates at dilution used

--- sample not obtained

Table IIB

Yellow chromogens on barley, flax, and soybeans during the growing season (x 10^4 /gm.)

1951	Barley		Flax		Soybeans	
	Stems and leaves	Heads	Stems and leaves	Heads	Stems and leaves	Pods
June 15	3[x]	---	0.8	---	19	---
June 22	61	---	0.1	---	25	---
July 7	0.8	---	0.1	---	4	---
July 16	0[xx]	---	0	---	0	---
August 3	59	53	---	---	---	---
August 9	100	219	192	278	3	---
August 20	36,957	1,876	1,286	134	166	---
September 6	1,042	467	58,928	1,200	2,194	---
September 17	55,363	2,327	78,947	8,065	2,284	381

x average of counts from four plates in each case

xx no count on any plates at the dilutions used

--- sample not obtained

Table IIIA

The epiphyte on wheat and oats during the growing season (x 10^4 /gm.)

1951	Redman Wheat		Thatcher Wheat		Oats	
	Stems and leaves	Heads	Stems and leaves	Heads	Stems and leaves	Heads
June 15	0[xx]	---	0.4[x]	---	0.8	---
June 22	12	---	0.2	---	0	---
July 7	0	---	0	---	0.01	---
July 16	0	---	0	---	0.8	---
August 3	4	578	0	15	51	0
August 9	192	2,477	215	190	172	54
August 20	17,007	485	9,524	7	311	194
September 6	12,069	1,382	10,925	928	9,063	10,000
September 17	40,115	15,243	45,137	3,777	13,769	3,571

x average of counts from four plates in each case

xx no count on any plates at the dilution used

--- sample not obtained

Table IIIB

The epiphyte on barley, flax and soybeans

during the growing season (x 10^4 /gm.)

1951	Barley		Flax		Soybeans	
	Stems and leaves	Heads	Stems and leaves	Heads	Stems and leaves	Pods
June 15	3[x]	---	0.8	---	19	---
June 22	61	---	0.05	---	17	---
July 7	0.8	---	0[xx]	---	0	---
July 16	0	---	0	---	0	---
August 3	8	12	---	---	---	---
August 9	67	219	192	228	3	---
August 20	24,638	866	900	134	119	---
September 6	1,042	333	41,250	600	1,567	---
September 17	41,522	1,551	55,263	5,043	1,634	298

x average of counts from four plates in each case

xx no count on any plate at the dilution used

--- sample not obtained

Table IVA

Incidence of yellow chromogens on wheat and oats expressed as percentages of the total bacterial populations

1951	Redman Wheat		Thatcher Wheat		Oats	
	Stems and leaves	Heads	Stems and leaves	Heads	Stems and leaves	Heads
June 15	0	---	5	---	6	---
June 22	39	---	40	---	33	---
July 7	0	---	0	---	10	---
July 16	0	---	0	---	40	---
August 3	4	89	20	50	11	57
August 9	11	81	14	75	28	60
August 20	64	41	50	18	27	80
September 6	24	24	33	27	65	63
September 17	60	50	50	38	66	69

Table IVB

Incidence of yellow chromogens on barley, flax and soybeans expressed as percentages of the total bacterial populations

1951	Barley		Flax		Soybeans	
	Stems and leaves	Heads	Stems and leaves	Heads	Stems and leaves	Pods
June 15	16	---	13	---	22	---
June 22	88	---	14	---	54	---
July 7	5	---	17	---	14	---
July 16	0	---	0	---	0	---
August 3	9	15	---	---	---	---
August 9	2	13	33	38	1	---
August 20	71	26	35	13	18	---
September 6	5	10	81	3	13	---
September 17	61	45	54	33	22	15

Table VA

Incidence of the epiphyte on wheat and oats expressed as percentages of yellow chromogens

1951	Redman Wheat		Thatcher Wheat		Oats	
	Stems and leaves	Heads	Stems and leaves	Heads	Stems and leaves	Heads
June 15	0	---	100	---	80	---
June 22	100	---	100	---	0	---
July 7	0	---	0	---	100	---
July 16	0	---	0	---	100	---
August 3	100	100	0	83	52	0
August 9	100	100	100	100	83	75
August 20	71	100	100	100	88	100
September 6	65	100	69	100	75	83
September 17	70	100	70	100	74	33

Table VB

Incidence of the epiphyte on barley, flax and soybeans expressed as percentages of yellow chromogens

1951	Barley		Flax		Soybeans	
	Stems and leaves	Heads	Stems and leaves	Heads	Stems and leaves	Pods
June 15	100	---	100	---	100	---
June 22	100	---	50	---	68	---
July 7	100	---	0	---	0	---
July 16	0	---	0	---	0	---
August 3	13	23	---	---	---	---
August 9	33	100	100	100	100	---
August 20	67	46	75	100	72	---
September 6	100	71	69	50	71	---
September 17	76	70	69	63	70	78

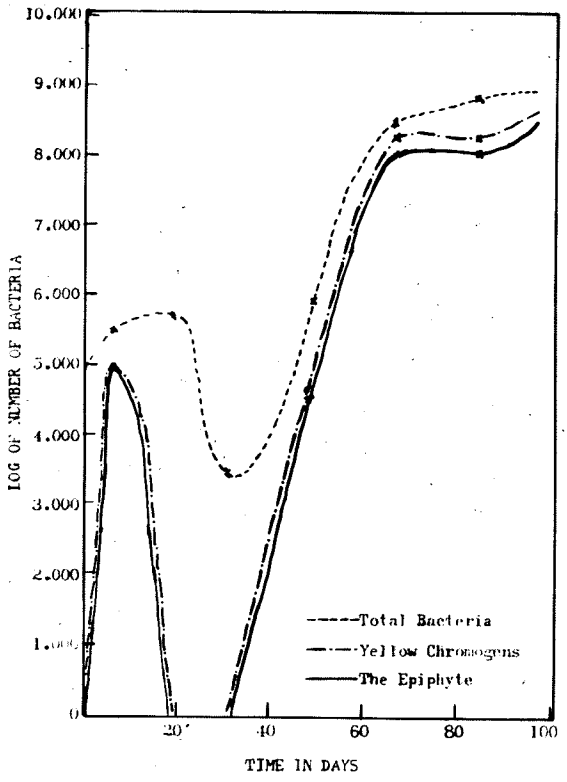

Fig. 1. Bacteria on Redman wheat (Stems and Leaves).

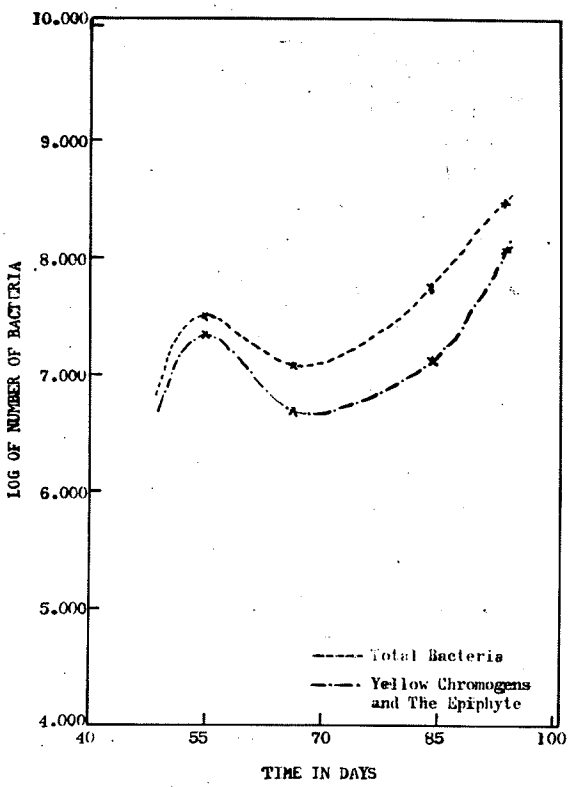

Fig. 2. Bacteria on Redman Wheat (Heads).

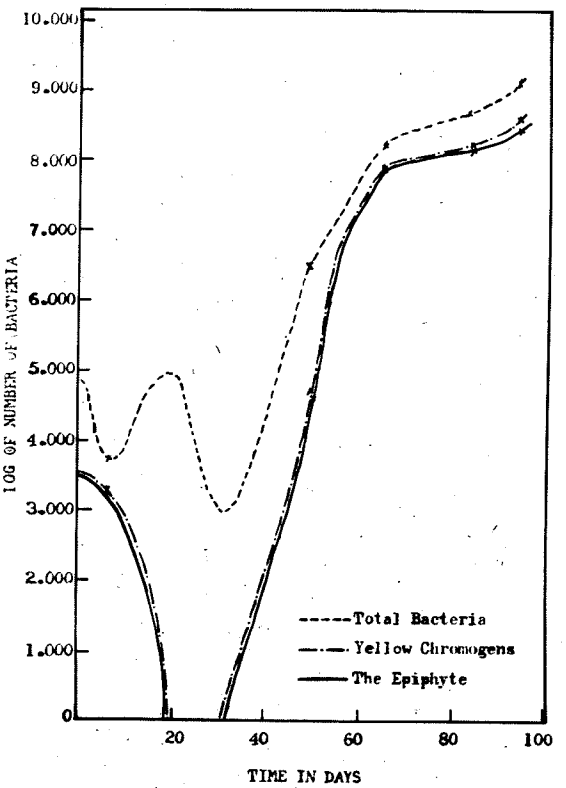

Fig. 3. Bacteria on Thatcher Wheat (Stems and Leaves).

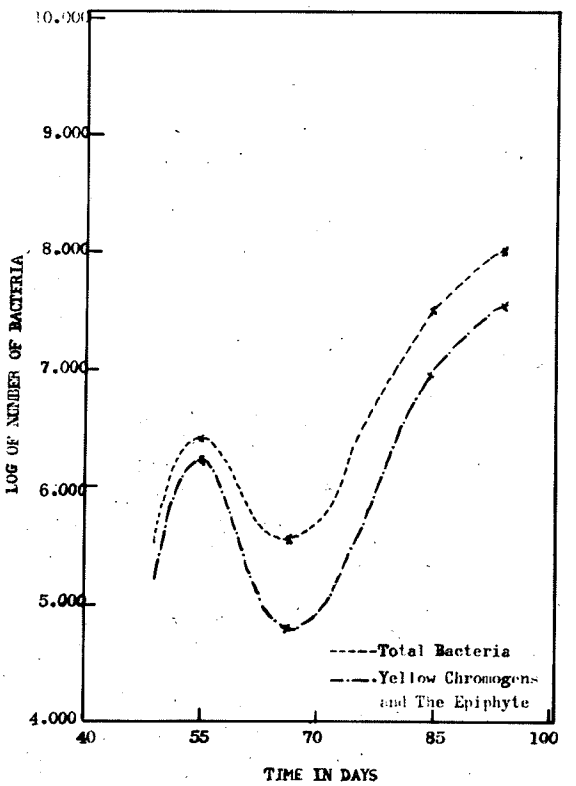

Fig. 4. Bacteria on Thatcher Wheat (Heads).

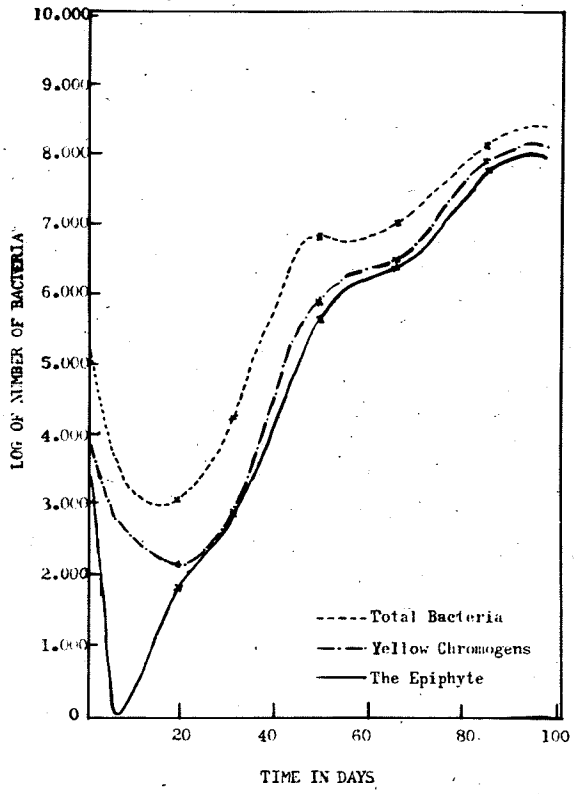

Fig. 5. Bacteria on Oats (Stems and Leaves).

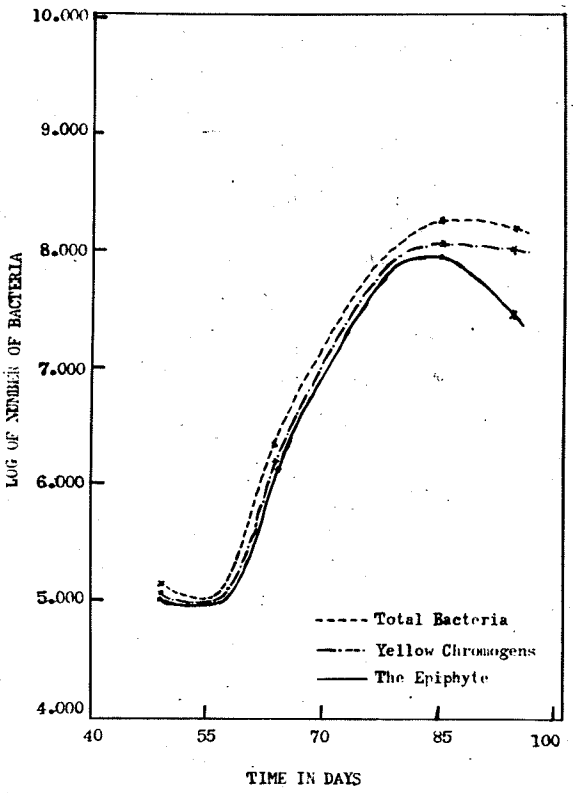

Fig. 6. Bacteria on Oats (Heads).

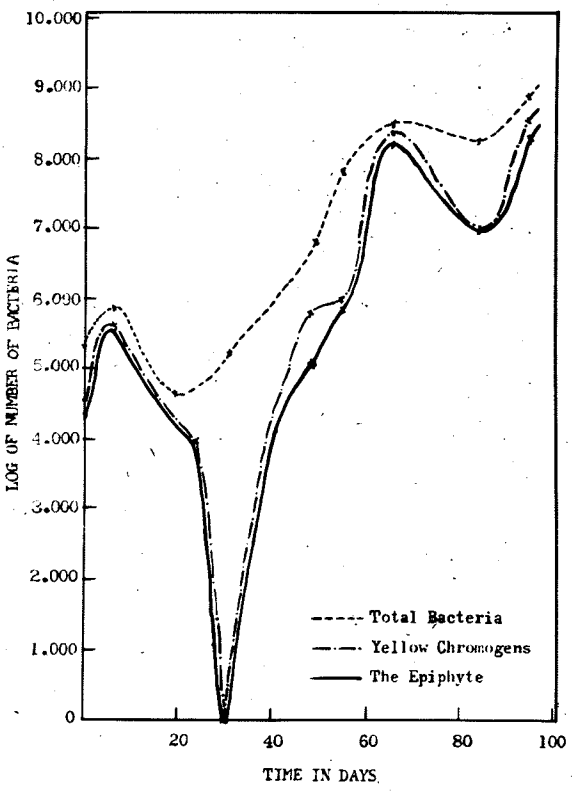

Fig. 7. Bacteria on Barley (Stems and Leaves).

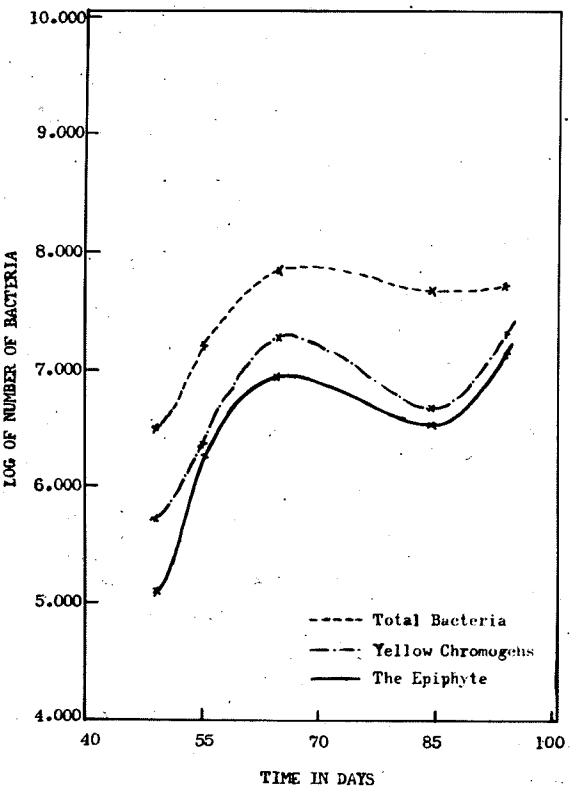

Fig. 8. Bacteria on Barley (Heads).

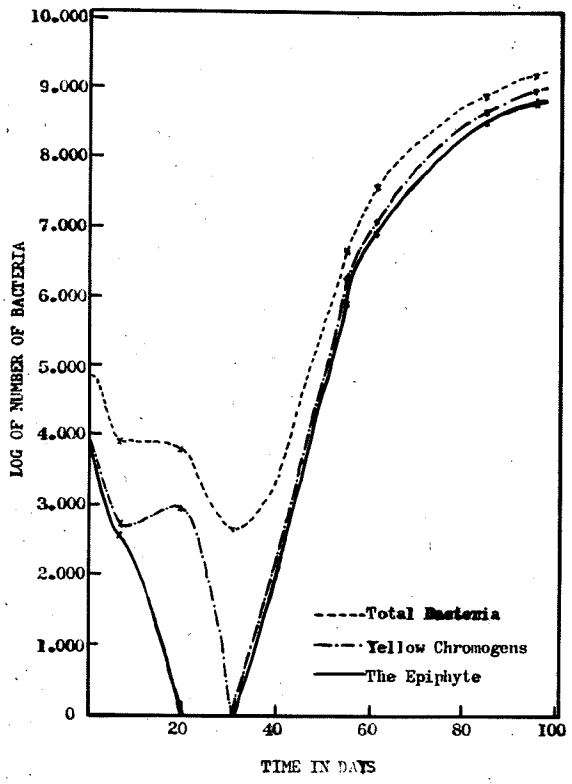

Fig. 9. Bacteria on Flax (Stems and Leaves).

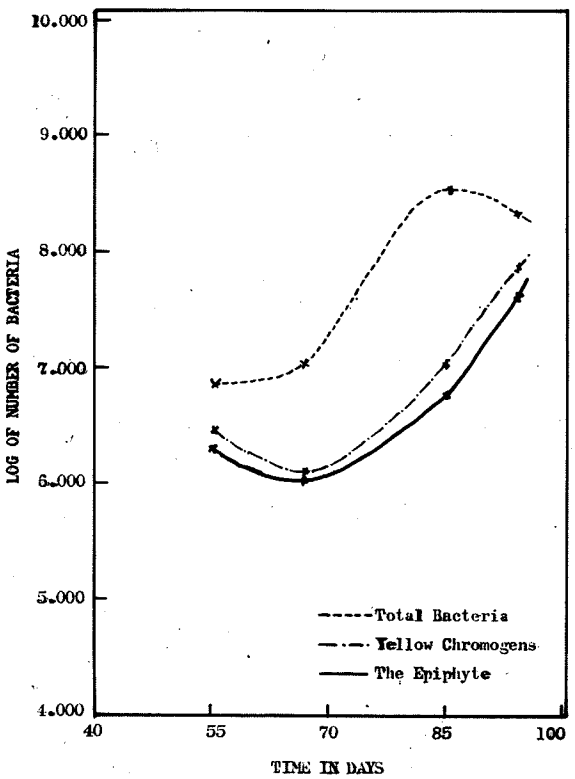

Fig. 10. Bacteria on Flax (Heads).

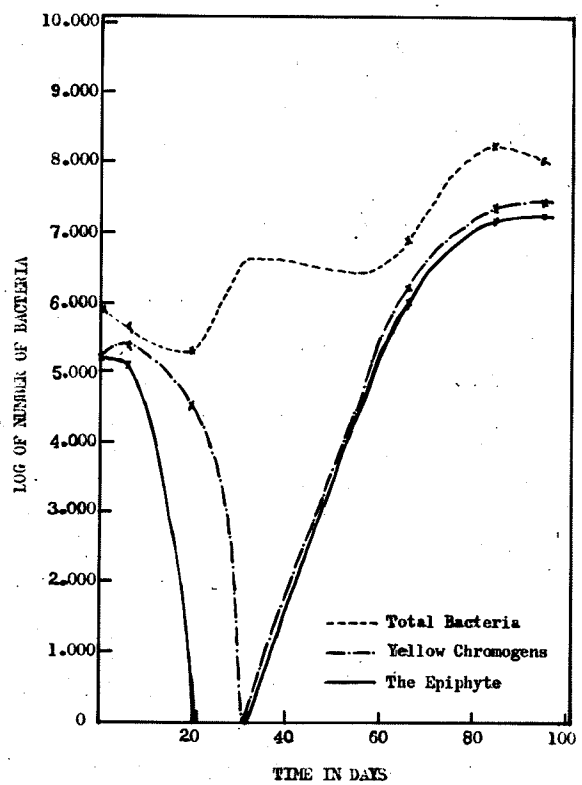

Fig. 11. Bacteria on Soybeans (Stems and Leaves).

PART II

YELLOW CHROMOGENS ON CERTAIN GRASSES AND SHRUBS

PART II

YELLOW CHROMOGENS ON CERTAIN GRASSES AND SHRUBS

The samples used in this part of the study were obtained on the campus at The University of Manitoba. They represented: lawn grasses, which will be referred to as Grass I and Grass II; honeysuckle leaves, and wild rose leaves. They were sampled at 0 (May 30, 1951), 7, 13, 38, 48, 104, and 135 days. The procedure followed was the same as outlined in Part I of this investigation.

The total count data, expressed as averages of counts on four plates from each sample, are recorded in Table VI. Counts of yellow chromogens are presented in Table VII; and of the epiphyte in Table VIII. The data, expressed as logarithms, are shown graphically in Figs. 12, 13, 14, and 15.

On the grasses the total bacterial population, and yellow chromogens, increased during the season, but to a smaller extent than on the grains referred to in Part I. Counts of the epiphyte were higly erratic throughout, but were a little more consistently high in late samplings.

All counts on the leaves of the two shrubs were much smaller than on the grasses, or on the grains referred to above. Yellow chromogens, and the epiphyte as well, were present on the shrub leaves but the counts were low.

Yellow chromogens likewise constituted a smaller proportion of the total bacterial population on these samples than on the grains; the

percentage being from 0 to 50. These results are presented in Table IX. As was the case with the grains, referred to in Part I, the epiphyte again comprised a large proportion of yellow chromogens. The epiphyte was the only yellow chromogen on 25% of the samples in this case. It constituted a larger proportion of yellow chromogens on the shrub leaves than on the grasses. The results are shown in Table X.

Table VI

Total bacteria on grasses, honeysuckle leaves and wild rose leaves during the growing season (x 10^4 /gm.)

1951	Grass I	Grass II	Honeysuckle leaves	Wild rose leaves
May 30	0[xx]	10,714[x]	0	67
June 6	24	8,626	8	113
June 12	112	13,000	0.8	516
July 7	9,756	4,113	1.0	109
July 17	85,034	100,000	0.6	21
September 11	55,169	26,328	79	351
October 12	262,840	298,883	48	295

x average of counts from four plates in each case

xx no count on any plate at the dilution used

Table VII

Yellow chromogens on grasses, honeysuckle leaves and wild rose leaves during the growing season (x 10^4 /gm.)

1951	Grass I	Grass II	Honeysuckle leaves	Wild rose leaves
May 30	0[xx]	1,205[x]	0	1
June 6	3	946	2	3
June 12	0	1,200	0.2	9
July 7	4,878	363	0.02	32
July 17	8,163	7,527	0.04	0.9
September 11	13,665	4,469	25	19
October 12	50,650	22,346	3	19

x average of counts from four plates in each case

xx no count on any plates at the dilution used

Table VIII

The epiphyte on grasses, honeysuckle leaves and wild rose leaves during the growing season (x 10^4 /gm.)

1951	Grass I	Grass II	Honeysuckle leaves	Wild rose leaves
May 30	0[xx]	0	0	0.7[x]
June 6	0.3	45	0.9	3
June 12	0	0	0.1	8
July 7	0	282	0.02	32
July 17	3,401	2,226	0.04	0.9
September 11	1,863	3,906	9	19
October 12	5,762	18,950	1	19

x average of counts from four plates in each case

xx no count on any plates at the dilution used

Table IX

Incidence of yellow chromogens on grasses,

honeysuckle leaves and wild rose leaves

expressed as percentages of total bacterial populations

1951	Grass I	Grass II	Honeysuckle leaves	Wild rose leaves
May 30	0	9	0	2
June 6	13	11	25	3
June 12	0	8	25	2
July 7	50	9	2	29
July 17	9	8	7	4
September 11	31	19	32	5
October 12	19	8	6	7

Table X

Incidence of the epiphyte on grasses,
honeysuckle leaves and wild rose leaves
expressed as percentages of yellow chromogens

1951	Grass I	Grass II	Honeysuckle leaves	Wild rose leaves
May 30	0	0	0	70
June 6	10	5	45	100
June 12	0	0	50	89
July 7	0	78	100	100
July 17	42	31	100	100
September 11	14	87	36	100
October 12	11	86	33	100

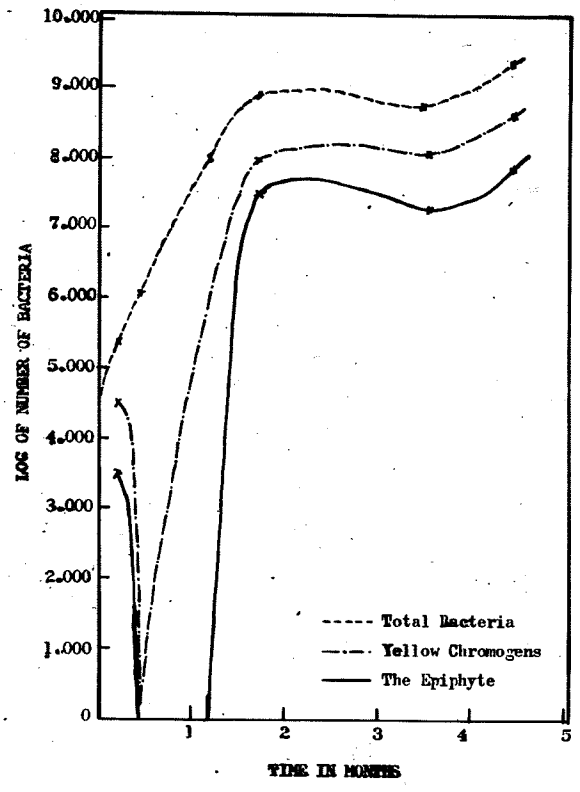

Fig. 12. Bacteria on Grass I.

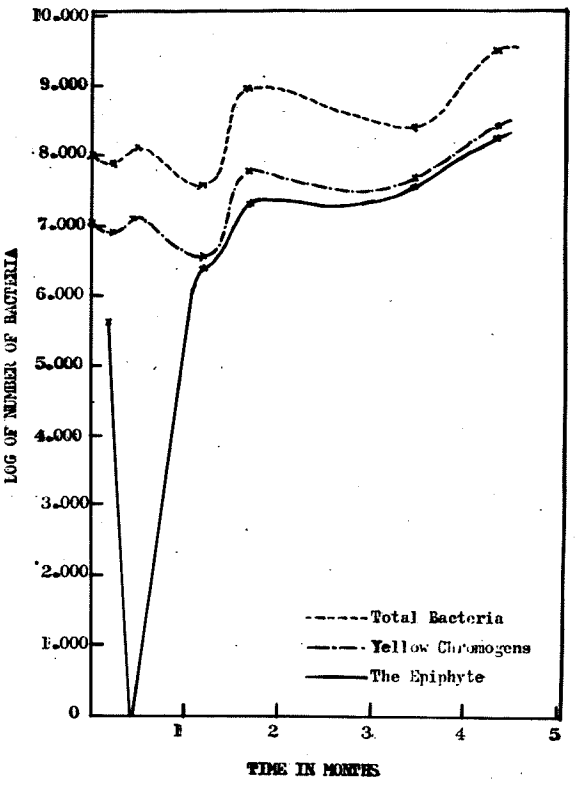

Fig. 13. Bacteria on Grass II.

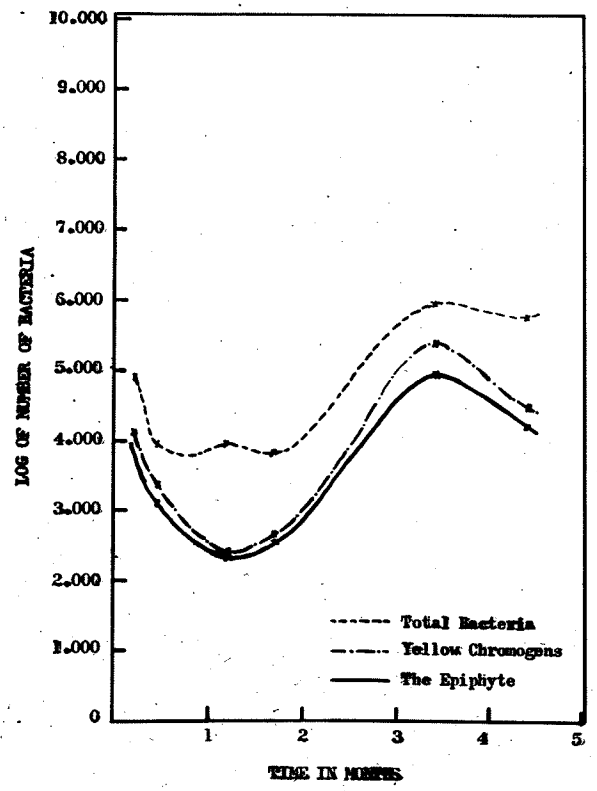

Fig. 14. Bacteria on Honeysuckle Leaves.

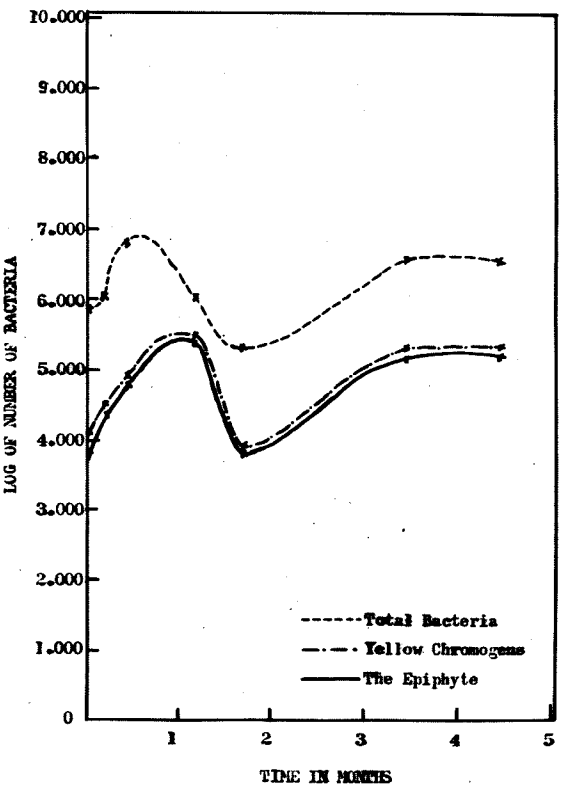

Fig. 15. Bacteria on Wild Rose Leaves.

PART III

YELLOW CHROMOGENS ON NORMAL AND GERMINATED WHEAT

PART III

YELLOW CHROMOGENS ON NORMAL AND GERMINATED WHEAT

Bacteria on normal wheat

Samples of Redman and Marquis wheats, provided through the courtesy of the Dominion Laboratory of Cereal Breeding located at The University, were used in this part of the study. The method employed for culturing the bacteria was that used by James et al (16). A 10-gm. sample of each wheat was prepared for plating by being diluted in 100-ml. sterile water in a 6-oz. screw-top bottle, containing as well 10-gm. sterile gravel, and shaken on a to-and-fro machine for 30 minutes. Appropriate dilutions were made; and duplicate plates were prepared using Difco nutrient agar as a substrate. Incubation was at 25°C. for 7 days.

The numbers of toal bacteria and of yellow chromogens were recorded and adjusted to a single-kernel basis. In order to distinguish the epiphyte the method outlined in Part I of this investigation was followed. The data on these samples are shown below.

Variety of Wheat	Total Bacteria per kernel	Yellow Chromogens per kernel	The Epiphyte per kernel
Redman	14,825*	2,426	1,078
Marquis	9,457	2,866	2,000

*--average of counts from four plates in each case

Yellow chromogens constituted 16% of the total bacteria on Redman wheat, and 30% on Marquis wheat, the epiphyte making up about 44% of these chromogens on Redman wheat, and about 70% on Marquis wheat.

Bacteria on germinated wheat

Replicate four kernel samples of each of Marquis and Redman wheats were germinated respectively in:-

(1) a beaker containing 100 gms. soil steam-sterilized at 121.5°C. for two hours

(2) a beaker containing 100 gms. natural soil

(3) a sterile Petri plate between two layers of moistened blotting paper

In order to determine the numbers of bacteria, yellow chromogens, and the epiphyte, each germinated kernel was plated by the procedure used on normal wheat samples.

Counts of total bacteria, yellow chromogens, and the epiphyte, adjusted as above to the single-kernel basis, are shown in Table XI.

Since counts of bacteria follow a Poisson distribution and not a normal distribution (2), a logarithmic transformation was applied to the counts of total bacteria to normalize the data. The analysis of variance on the transformed data follows:-

Source of Variation	D. F.	Mean Square	F	$F_{.05}$	$F_{.01}$
Replicates	3	.0174	2.42	9.28	29.46
Whole plot treatments (Varieties)	1	.2095	29.09*	10.13	34.12
Error for Varieties	3	.0072			
Total for Varieties	7				
Split-plot treatments (methods of germination)	3	15.932	698.80**	3.16	5.09
Interaction (varieties x methods)	3	.3395	14.89**	3.16	5.09
Error for methods	18	.0228			
Total for methods	24				
Total	31				

By applying the formula $t = \frac{\text{difference in means}}{\text{standard error}}$

where standard error $= \frac{2 \times \text{Error mean square for methods of germination}}{\text{number of replicates}}$

the following t values were calculated.

Differences in means of numbers of bacteria on normal grain and when germinated in three different ways	t	$t_{.05}$	$t_{.01}$
Redman			
in natural soil	23.76**	2.10	2.88
in sterile soil	32.37**	2.10	2.88
between two layers of moistened blotting paper in a Petri plate	21.32**	2.10	2.88
Marquis			
in natural soil	26.08**	2.10	2.88
in sterile soil	27.39**	2.10	2.88
between two layers of moistened blotting paper in a Petri plate	25.26**	2.10	2.88

The total bacteria were higher on germinated Redman and Marquis kernels than on normal kernels. From the above analysis of variance and t - test, it is apparent that the difference in counts of total bacteria was highly significant regardless of method of germination.

Yellow chromogens, and the epiphyte likewise, were higher on germinated kernels, than on normal kernels (Table XI). The proportion of yellow chromogens to total bacteria varied appreciably, in Redman wheat averaging 65% in kernels germinated in sterile soil and 7½% in kernels germinated in natural soil. These higher values for kernels

germinated in sterile soil did not hold true for the sample of Marquis wheat where proportions on kernels in natural soil were slightly higher. These data are presented in Table XII. However, on both varieties the epiphyte constituted approximately the same proportion of yellow chromogens on both normal and germinated kernels. These results are shown in Table XIII.

Table XI

Total bacteria, yellow chromogens, and the epiphyte on germinated wheat (x 10^4 /kernel)

Variety of grain and method of germination	Replicates	Total Bacteria	Yellow Chromogens	The Epiphyte
Redman germinated in natural soil	1	440[x]	50	50
	2	1,030	110	20
	3	545	20	10
	4	234	9	3
Redman germinated in sterile soil	1	3,925	2,500	750
	2	4,000	2,500	2,000
	3	3,700	2,500	2,000
	4	4,435	2,500	1,000
Redman germinated between pieces of moistened, sterile blotting paper	1	218	8	3
	2	253	10	4
	3	230	100	90
	4	420	100	60
Marquis germinated in natural soil	1	296	100	100
	2	630	90	80
	3	565	50	30
	4	865	104	87
Marquis germinated in sterile soil	1	780	130	120
	2	705	90	60
	3	915	120	80
	4	652	98	66
Marquis germinated between pieces of moistened, sterile blotting paper	1	405	70	60
	2	675	110	70
	3	555	80	20
	4	271	125	125

x average of counts from four plates in each case

Table XII

Incidence of yellow chromogens

on normal and germinated wheats

expressed as percentages of total bacterial populations

Variety of Wheat	Normal kernel	Method of germination	Replicate kernels 1	2	3	4
Redman	16[x]	natural soil	11	11	4	4
		sterile soil	64	63	68	56
		between pieces of moistened, sterile blotting paper	4	4	43	24
Marquis	30[x]	natural soil	34	14	9	12
		sterile soil	17	12	13	15
		between pieces of moistened, sterile blotting paper	17	16	14	46

x based on average per kernel in the 10-gm. sample used for plating

Table XIII

Incidence of the epiphyte

on normal and germinated wheats

expressed as percentages of yellow chromogens

Variety of Wheat	Normal kernel	Method of germination	Replicate kernels 1	2	3	4
Redman	44[x]	natural soil	100	9	50	33
		sterile soil	30	80	80	40
		between pieces of moistened, sterile blotting paper	38	40	90	60
Marquis	70[x]	natural soil	100	89	60	84
		sterile soil	92	67	67	67
		between pieces of moistened, sterile blotting paper	86	64	25	100

x based on average per kernel in the 10-gms sample used for plating

PART IV

YELLOW CHROMOGENS ON CLOVER HAY

PART IV

YELLOW CHROMOGENS ON CLOVER HAY

The bacterial flora on clover hay was investigated using the procedure outlined in Part I, that is, counts of total bacteria, yellow chromogens, and the epiphyte were obtained. These are shown below.

Sample of Clover Hay	Total Bacteria ($\times 10^4$ /gm.)	Yellow Chromogens ($\times 10^4$ /gm.)	The Epiphyte ($\times 10^4$ /gm.)
A	18,987*	717	422
B	20,492	1,257	929
C	19,231	385	289

*--average of counts from four plates in each case

Yellow chromogens constituted a smaller proportion of the total bacteria on clover hay than on the grains, grasses or shrubs, referred to in Parts I and II; the percentages being four, six, and two, respectively. The epiphyte made up about 59, 69, and 74% of the yellow chromogens on these samples.

According to Bergey's Manual (4), Pseudomonas trifolii Huss was isolated originally from clover hay, and is a common organism on the leaves of plants. This species resembles the epiphyte in most characteristics. However, it differs in two respects. Pseudomonas trifolii

produces indole from tryptophane broth and cultures have an agreeable odor. The epiphyte exhibits neither of these characteristics. In order to determine whether any of the yellow chromogens isolated represented Pseudomonas trifolii, each was tested further for:

(1) Production of indole

Duplicate tryptophane broth cultures, incubated at 25°C. for 3 days, were tested by the Gnezda oxalic acid test (6); and a second pair of cultures by Kovac's modification of the Ehrlich Bohme test. This test was performed by adding 2 or 3 drops of the following reagent:-

p - dimethylaminobenzaldehyde	5 gms.
Amyl alcohol	75 gms.
Concentrated hydrochloric acid	25 gms.

to each of the cultures. A positive test was indicated by the appearance of a bright red color.

None of the isolates produced indole. Nor did the culture of Steel (33), which was used as a check in this study. At each testing, Escherichia coli and Proteus vulgaris cultured in the same medium regularly produced indole, which provided a check on methods.

(2) Production of an agreeable odor

None of the yellow chromogens in this study produced a characteristic odor on any of the media used.

DISCUSSION

DISCUSSION

The finding of increasing numbers of yellow chromogens and of the epiphyte on grains during the growing season confirms the results of Stark (30). However, the numbers reported in this study are not directly comparable with those stated by Stark since estimates in the previous study were made on the basis of area of leaf surface.

Since yellow chromogens and the epiphyte were found on grains early in the season, it is difficult to account for their absence on samples obtained on July 7 and 16. Perhaps climatic conditions, such as temperature and moisture at the time of sampling, might have had an effect on numbers. However, since no record of climatic conditions during the investigation was kept, no inference from this probability can be drawn, although it is significant that low counts on shrubs and grasses likewise were obtained on samples plated about the same time. Of course, the absence of bacteria on plates prepared on these dates should not be interpreted as meaning absolute freedom from bacteria. It is conceivable that on these plants numbers were sufficiently low not to appear in the lowest dilutions plated, but still present in numbers sufficiently high to allow for reproduction later when conditions became more favourable; which was obviously the basis of the higher counts obtained about the time of heading.

Such high counts of yellow chromogens and of the epiphyte on all plants, recorded later in the season, might be due to either of two factors. Firstly, physiological changes within the plant as it reaches

maturity may be such as to provide a better medium for these types. Of course, in the case of grasses which were cut periodically the same evidence of maturity was not apparent. Undoubtedly, if the grasses had been allowed to grow uninterruptedly the same result would have been obtained. Secondly, climatic conditions at ripening time would favour probably the increase of these bacteria.

Since physiological changes within the seed probably favour the rapid multiplication of bacteria on the seed and hence influence the actual count, it is not surprising that such a significantly higher bacterial population was present on germinated kernels. Mack's (22) results on sprouted and unsprouted wheats were somewhat similar to those obtained in this study. Her counts, based on an average of 20 kernels, were 60,000 bacteria per kernel of unsprouted wheat and 1,300,000 bacteria per kernel of sprouted wheat. However, counts in these two studies may not be directly comparable. Presumably, Mack plated samples that had germinated naturally; and her technique differed in certain minor details.

The moisture and temperature conditions provided in these germination studies undoubtedly were much the same as would be found when seeds are incubated on nutrient agar in a Petri plate. Consequently, the suppressing effect of Helminthosporium sativum P. K. & B. in nutrient agar, as reported by Simmonds (27), probably could be attributed to competition for nutrients between epiphyte and pathogen, rather than to the production of an antagonistic substance by the epiphyte.

Strangely, even though yellow chromogens made up a large portion of flora on clover hay none of the isolates studied proved to be Pseudomonas trifolii Huss, whereas the epiphyte constituted a considerable proportion of the isolates. It should be noted, however, that the samples used in this study represented cured hay, rather than freshly-cut hay or growing clover.

SUMMARY

SUMMARY

(1) Samples of the following grains:- Redman wheat, Thatcher wheat, Exeter oats, Montcalm barley, Dakota flax, and Pagoda soybeans, grown at The University of Manitoba, were plated during the growing season. Numbers of total bacteria, yellow chromogens, and of a type of yellow chromogen reported previously by others to be an epiphyte, were small at the first four samplings and showed little evidence of a consistent trend. Later, numbers increased appreciably and remained at a high level at all samplings. Yellow chromogens constituted from 0 to 88% of the total bacterial population. The epiphyte was the only yellow chromogen on 38% of the samples and made up a large proportion on most of the others.

(2) Using the same procedure as for the grains, samples of lawn grasses, honeysuckle leaves, and wild rose leaves were plated during the growing season. Counts of total bacteria, yellow chromogens, and the epiphyte increased during the season but to a smaller extent and with less consistency than on the grains. From 0 to 50% of total bacteria on grasses and shrub samples were yellow chromogens. The epiphyte was the only yellow chromogen on 25% of the samples and again, as in the case of the grains, constituted a large proportion on the others.

(3) Germinated kernels of Redman and Marquis wheats, whether germinated in natural soil, in sterile soil, or between pieces of moistened, sterile, blotting paper, produced significantly higher counts of

total bacteria than did normal samples of the same varieties of wheat. Likewise, yellow chromogens were higher on germinated kernels than on normal kernels, but the proportion of yellow chromogens to total bacteria was not the same on germinated kernels as on normal kernels. However, the epiphyte represented about the same proportion of yellow chromogens on both normal and germinated kernels.

(4) The epiphyte made up 59, 69, and 74% of yellow chromogens on three samples of clover hay, while the yellow chromogens, in turn, constituted four, six, and two percent of total bacteria on these same samples. None of the yellow chromogens from clover hay were representative of Pseudomonas trifolii Huss.

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