CERTAIN STUDIES ON
A SALTANT OF
HELMINTHOSPORIUM SATIVUM

by

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INTRODUCTION

Helminthosporium sativum P.K. & B. has been recognized as being an important species associated with the root-rot disease of cereals (3) (12). Since this pathogen is regarded as soil-borne (9), it appeared reasonable to assume that some soil complex might exert an influence upon its development.

A fertile saltant of this species produced by Greaney and Machacek (11) was used. It differs from the parent in that the mycelium is almost colorless and the conidia are hyaline. This strain has retained the pathogenicity of the parent and seemingly has remained stable in almost all respects since its production in 1930[1]. Further, it develops a faint salmon pink color when cultured on Czapek's agar at pH 4.5 to 5.5. It was used in this study primarily because of the ease of identification on this medium.

The investigation was divided into two parts.

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[1]Personal communication from Dr. J. E. Machacek.

PART I

SURVIVAL OF THE SALTANT IN CERTAIN SOILS

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Field-plot experiments by Greaney and Machacek[1] have shown that after heavy inoculations of a saltant of *Helminthosporium sativum* P. K. & B. into soil the disease incidence in the initial crop was high while in the subsequent crop, the incidence was very low. It appears logical to assume that a large proportion of the original inoculum failed to survive during the period between crops. In order to get information on this point an investigation was carried out under laboratory conditions using this saltant in different soils.

Only two references dealing with the survival of other strains of this species appear to be available. Christensen (4) showed that various strains of *Helminthosporium sativum* P. K. & B. overwintered readily in soil and on decaying plant remains in both the conidial and mycelial stages. He showed further, that conidia viability in soil was greatly influenced by environment. Percentage germination of spores buried in soil varied from 2.5 to 81.5 per cent in three consecutive years. Katznelson (15) showed that a strain of *Helminthosporium sativum* P. K. & B. inoculated into sterile soil increased to about 40

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[1]Personal communication from Dr. J. E. Machacek.

times the original number during the first 14 days and then decreased gradually. At the end of 61 days, the numbers were reduced to a point still four times as great as the initial number.

EXPERIMENTAL

Samples representing soils ranging in texture from loamy sand to clay were obtained from six cultivated fields about 40 miles east of Winnipeg. Each was a composite of four subsamples. Each was stored at room temperature in the laboratory for several weeks. The moisture equivalent of each sample was determined (2). These data are presented in Table I. Then enough of each sample was transferred to each of twenty 50 ml. Erlenmeyer flasks to make 25 gm. when brought to the moisture equivalent of the soil by the addition of the required amount of inoculum and sterile water. One half of the replicate samples was heated at 15 pounds steam pressure for four hours on each of two consecutive days. This sterilizing procedure represented a longer exposure to a high temperature than was used by Katznelson (15). The other half of the replicates was used in the raw state to provide data on the effect of the normal soil flora on the survival of the pathogen. A 2.0 ml. aliquot of a uniform suspension in sterile water of conidia and fragments of mycelium of the saltant and sufficient extra sterile water to make up to 25 gm. were added to each flask. The suspension was prepared by washing 14-day-old Czapek's agar cultures with sterile distilled water. Its density was determined by the Neubauer haemocytometer slide method (16).

Each flask was fitted with a one-hole rubber stopper in which a glass tube having a bore of 2.5 mm. was inserted. The tube was plugged loosely with cotton. The moisture loss over the period of the investigation is shown in Table II. In order to prevent contamination by fungi growing into the flask around the stopper, melted paraffin was applied to the stopper and to the neck of the flask. Samples were incubated at 25° C. Counts of fungi and of bacteria were made on one replicate of each of the raw and the treated samples at the beginning and on other replicates at weekly intervals. Sodium albuminate agar medium was used for bacteria and Czapek's agar medium, plus 0.5 ml. of 10% lactic acid per 100 ml. medium, for fungi. The results are presented in Table III and graphically in Fig. 1.

Table I. Moisture equivalents and amounts of water added to make 25 gm. samples.

Soil	Moisture equivalent	water added
1	14.8%	2.76 ml.
2	47.2	6.46 "
3	30.4	4.97 "
4	56.8	7.75 "
5	30.0	5.55 "
6	42.8	6.14 "

Table II. Moisture loss by weeks expressed as a percentage of the 25 gm. sample of moist soil.

Soil	1	2	3	4	5	6	7	8	9
1S	0.04	0.08	0.12	0.20	0.24	0.32	0.44	0.48	0.48
1	0.04	0.08	0.12	0.20	0.24	0.36	0.40	0.44	0.48
2S	0.04	0.12	0.16	0.24	0.28	0.36	0.40	0.48	0.52
2	0.04	0.12	0.24	0.28	0.32	0.36	0.40	0.44	0.52
3S	0.08	0.12	0.16	0.16	0.20	0.32	0.32	0.40	0.44
3	0.04	0.08	0.12	0.20	0.24	0.28	0.32	0.40	0.44
4S	0.00	0.04	0.08	0.16	0.24	0.32	0.32	0.36	0.48
4	0.08	0.16	0.20	0.20	0.24	0.24	0.32	0.36	0.44
5S	0.04	0.08	0.12	0.16	0.24	0.28	0.40	0.44	0.52
5	0.04	0.12	0.16	0.16	0.20	0.24	0.28	0.32	0.36
6S	0.04	0.04	0.08	0.20	0.24	0.24	0.32	0.44	0.48
6	0.08	0.08	0.12	0.20	0.20	0.32	0.36	0.40	0.48

The soil designated 1S is the same as that designated 1 except that it was sterilized.

Table III. Survival of a saltant of *H. sativum* in raw and sterilized soils.

Soil		raw			sterilized		
	weeks	saltant X10^3	fungi X10^3	bact. X10^5	saltant X10^3	fungi X10^3	bact. X10^5
1.	0	61.0	27.5	270.0	66.0	----	----
	1	15.0	20.0	260.0	60.0	----	----
	2	5.0	15.0	245.0	50.0	----	----
	3	0.5	15.0	225.0	42.75	----	----
	4	0.0	17.5	235.0	45.0	----	----
	5	0.0	15.0	225.0	42.0	----	----
	6	0.0	16.0	210.0	42.5	----	----
	7	0.0	14.5	230.0	38.0	----	----
	8	0.0	16.0	220.0	40.0	----	----
	9	0.0	15.0	205.0	43.75	----	----
2.	0	67.5	22.5	160.0	30.0	----	----
	1	11.0	20.0	215.0	55.0	----	----
	2	3.0	20.0	210.0	43.5	----	----
	3	2.0	18.5	205.0	25.0	----	----
	4	0.25	25.0	215.0	20.0	----	----
	5	0.0	25.0	240.0	17.5	----	----
	6	0.0	20.0	230.0	14.5	----	----
	7	0.0	18.5	210.0	16.0	----	----
	8	0.0	21.0	190.0	10.0	----	----
	9	0.0	17.5	175.0	8.5	----	----

Table III. Cont.

Soil	weeks	raw saltant X10^3	raw fungi X10^3	raw bact. X10^5	sterilized saltant X10^3	sterilized fungi X10^3	sterilized bact. X10^5
3.	0	73.5	32.5	320.0	56.5	----	----
	1	20.0	25.0	305.0	36.0	----	----
	2	3.5	15.0	280.0	42.5	----	----
	3	1.0	15.0	275.0	50.0	----	----
	4	0.0	20.0	255.0	20.0	----	----
	5	0.0	17.5	220.0	12.0	----	----
	6	0.0	15.0	200.0	8.0	----	----
	7	0.0	16.0	215.0	7.0	----	----
	8	0.0	14.0	185.0	7.0	----	----
	9	0.0	12.5	200.0	9.5	----	----
4.	0	77.5	22.5	185.0	60.0	----	----
	1	50.0	20.0	240.0	38.0	----	----
	2	2.0	15.0	170.0	35.0	----	----
	3	1.5	10.0	175.0	37.0	----	----
	4	0.0	8.0	185.0	34.0	----	----
	5	0.0	6.0	175.0	30.0	----	----
	6	0.0	5.0	195.0	25.0	----	----
	7	0.0	7.5	185.0	24.0	----	----
	8	0.0	5.0	160.0	25.5	----	----
	9	0.0	6.0	145.0	19.0	----	----

Table III. Cont.

Soil	weeks	raw saltant X10^3	raw fungi X10^3	raw bact. X10^5	sterilized saltant X10^3	sterilized fungi X10^3	sterilized bact. X10^5
5.	0	60.0	20.0	370.0	50.0	----	----
	1	32.5	35.0	380.0	35.0	----	----
	2	2.5	20.0	225.0	26.25	----	----
	3	1.25	15.5	240.0	15.0	----	----
	4	0.0	18.5	220.0	12.5	----	----
	5	0.25	15.0	200.0	8.5	----	----
	6	0.0	12.5	175.0	6.0	----	----
	7	0.0	14.5	155.0	4.5	----	----
	8	0.0	12.5	130.0	7.0	----	----
	9	0.0	14.0	150.0	5.5	----	----
6.	0	40.0	80.0	415.0	42.5	----	----
	1	42.5	50.0	400.0	28.5	----	----
	2	6.0	20.0	360.0	20.0	----	----
	3	0.5	16.25	350.0	15.5	----	----
	4	0.0	20.0	335.0	18.5	----	----
	5	0.0	18.5	315.0	15.0	----	----
	6	0.0	20.0	290.0	12.5	----	----
	7	0.0	20.0	260.0	14.5	----	----
	8	0.0	16.0	280.0	12.5	----	----
	9	0.0	14.0	305.0	14.0	----	----

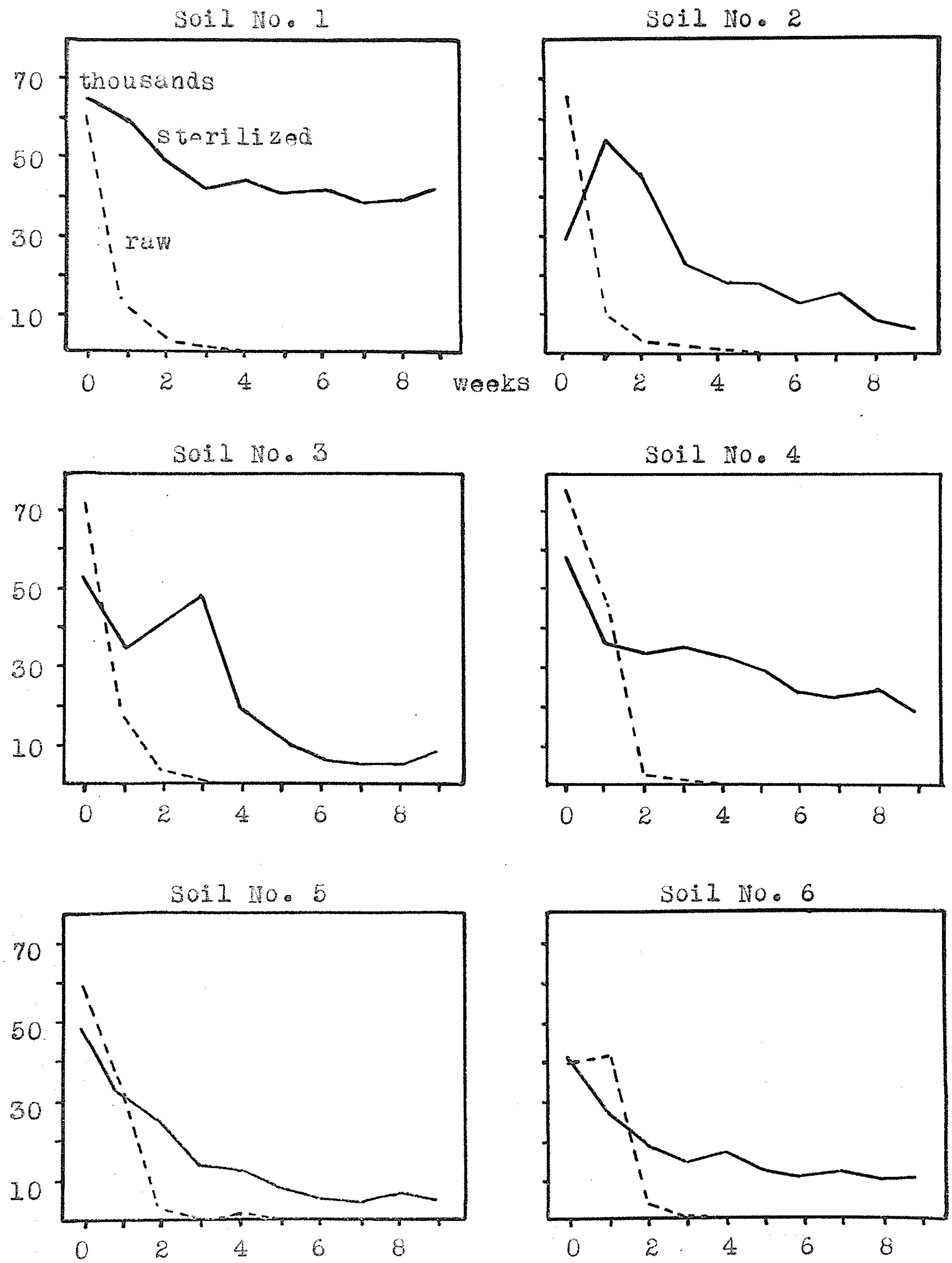


Fig. 1 Survival of a saltant of H. sativum in raw and sterilized soils.

DISCUSSION

It was considered advisable that the soils used in a study of this nature should represent a fairly wide range in texture. The variation in texture of these six soils is evidenced by the difference in moisture equivalents reported in Table I.

The procedure followed in adjustment for moisture failed to take into consideration the probable loss of moisture during the long period of sterilization. This loss may have been different in the six soils studied. Consequently, the moisture may not have been at the optimum for the saltant. Under field conditions, the disappearance of the saltant might be associated in part with changes in moisture and temperature. In this study, however, the moisture loss was small, as is shown in Table II, and the temperature was constant at 25° C. These facts would seem to indicate that the reduction in numbers of the saltant could scarcely be explained on the basis of change in moisture and/or temperature.

Katznelson (15) reported that a strain of <u>Helminthosporium sativum</u> P. K. & B. showed a higher number at the seventh and fourteenth days and then declined slowly. At the end of 61 days the count was still four times that of the initial count. The saltant used in this study did not show a similar trend. In four of the sterilized soils used in this study, the numbers declined steadily during the nine week period.

The remaining two soils showed a slight increase at first but this was followed by the gradual decline noted in the other sterilized soils. The initial increase noted in the two soils did not appear to have a consistent relationship to the ability to hold moisture. The two that showed increases in numbers had moisture equivalents that ranked second and third in the six soils investigated. In no case was the final number at the end of nine weeks as high as the initial number.

Certain theories might be advanced to explain the decline in numbers in sterilized soils. The products of staling could possibly, in time, reach a sufficiently high concentration that growth would be inhibited. The possibility of exhaustion of food might also be considered, particularly with a large inoculum.

In the raw soils the decline in numbers was rapid. At the end of 14 days the decrease in numbers ranged from 85.0 per cent in the case of soil #6 to 97.4 per cent in the case of soil #4. In no case was the saltant recovered after the fifth week. The factors responsible for the small counts in sterilized soils may have affected the results in raw soils. In addition, it may be possible that the saltant could not compete with the other fungi and bacteria present in respect to nutrients. As well, the production of growth-inhibiting substances by various soil fungi and bacteria are well known (18); and it may be that the survival of the saltant was affected by these products.

A major difficulty in estimating numbers of this saltant in raw soils lies in the fact that this strain does not develop on Czapek's agar as readily as do various other soil fungi. This may mean that although the strain was present at a given dilution, the early development of other species would reduce the numbers of this saltant on the plates counted. This would seem to apply particularly at low dilutions.

The plate method of counting may not be reliable for determining survival of this saltant. The plate count may be accepted as giving, primarily, a measure of viable inoculum units (spores plus fragments of mycelium). Conditions in the different soils may have produced differences in the vegetative growth and in the sporulation of the saltant that were not reflected in the plate count.

This evidence of reduction of the saltant in soil would appear to provide the reason for the decline of the incidence of the disease obtained by Greaney and Machacek.

SUMMARY

1. An investigation was carried out to determine the survival of a white saltant of <u>Helminthosporium sativum</u> P. K. & B. in raw and sterilized portions of six different soils.

2. Numbers of the saltant declined slowly in four of the sterilized soils. In the two remaining sterilized soils, the numbers increased at first but then declined gradually. In no case was the final number as great as the initial number.

3. Numbers of the saltant declined rapidly in all of the raw soils used. The saltant was not recovered from any of the raw soils after the fifth week.

PART II

METHODS OF COUNTING

PART II

METHODS OF COUNTING

The quantitative estimation of bacterial and fungal populations has been conducted, in general, along two main lines, - (1) the plate count method and (2) the direct microscopic count method.

The plate count method has been studied in recent years by a number of investigators. Brierley et al (1) discussed the method and its limitations with respect to soil fungi and conclude with the statement:

> "If an impeccable and standardized technique be adopted replicated experiments give numbers of a most satisfactory degree of uniformity."

Fisher et al (7) and James and Sutherland (14) investigated the method with respect to its mathematical reliability. While these investigators have not suggested that the plate count method is without limitations, they have shown that it has definite value for comparing two or more samples.

The direct microscopic count method has been used by various investigators. Conn (5) applied the basic principle of directly counting the number of cells in a given volume in his studies on soil bacteria. Horsfall (13) outlined a modification of this procedure in his studies on fungicides. The haemocytometer slide was originally designed for the direct microscopic counting of blood cells.

Student (17) in 1906 conducted an investigation on the mathematical error arising from the use of this type of slide. More recently the haemocytometer slide has been used for the quantitative determination of fragments of fungal mycelium and spores in certain food products (16).

The purpose of this investigation was to compare estimates obtained by (1) the plate count method, and (2) the direct microscopic count method using the Neubauer haemocytometer slide.

EXPERIMENTAL

Each suspension of the saltant was prepared by introducing 10 ml. of sterile water into a three oz. bottle-slant-culture grown on Czapek's agar at 25° C. for 14 days; and by removing the growth with a sterile needle. This was shaken 25 times immediately before counts were made. Using the Neubauer haemocytometer slide method, counts of (a) spores alone and (b) spores plus fragments of mycelium were made on five 0.0001 ml. portions on each of four slides. The data are presented in Table IV.

In order to test the validity of an estimate on one slide based on the count from one field, a value of X^2 (chi square) was calculated from the five counts on each of 100 slides by the equation of Fisher et al (7):

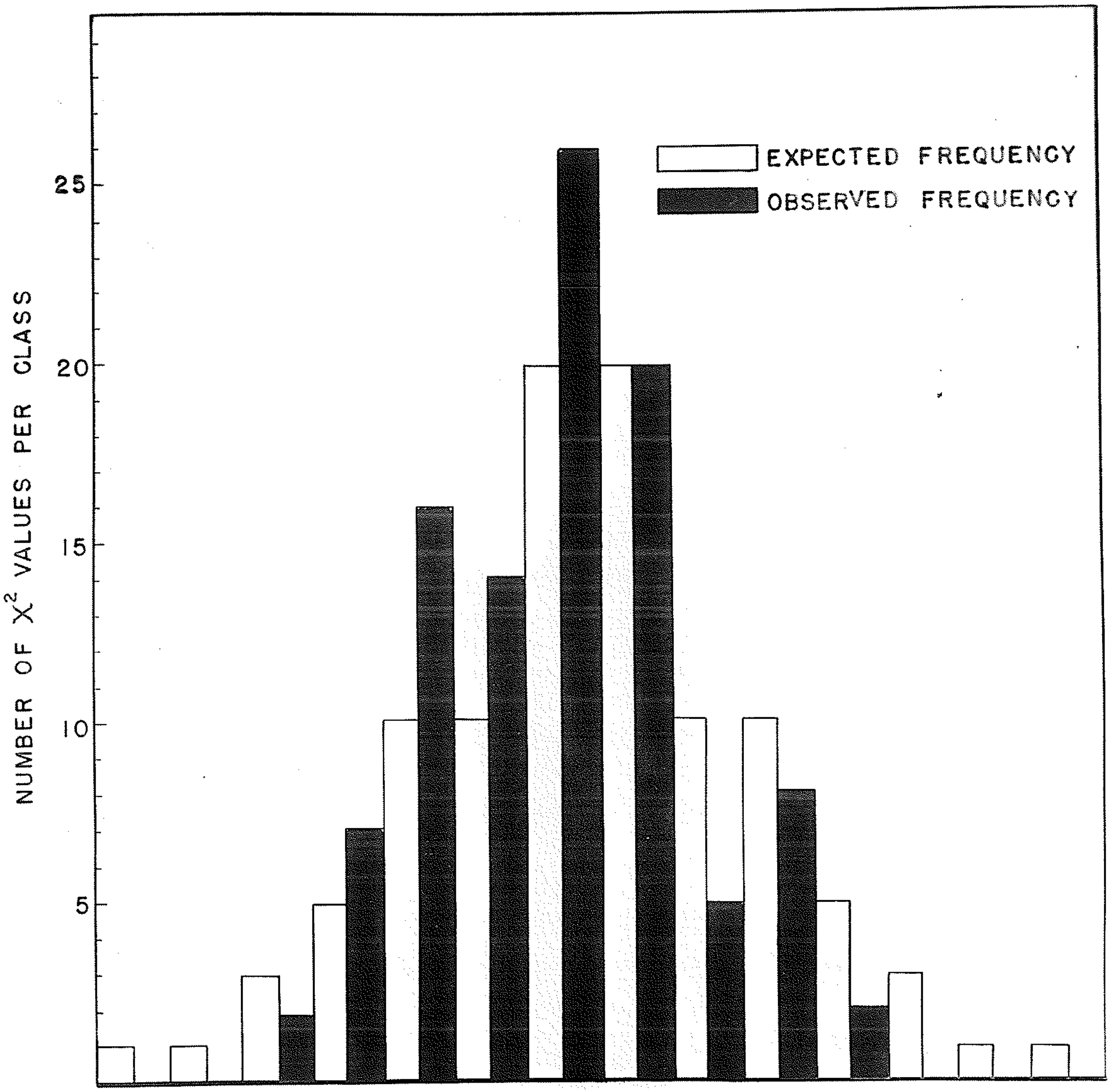
$$X^2 = \frac{S\,(x - \bar{x})^2}{\bar{x}}$$

where x is the number of units counted in each field and $\bar{x}$ is the mean of the set. The 100 chi square values thus obtained were arranged in classes using the method of Fisher et al (7); and the observed frequency was compared with the theoretical or expected frequency by a Goodness of Fit test (6). These results are presented in Fig. 2. The P value obtained, 0.09, (8) was accepted as evidence of the validity of an estimate based on one field. Further, as evidence of the reliability of an estimate on a suspension based on the total count from one slide, a X^2 value was calculated from the counts on four slides from one suspension. These X^2 values were distributed into classes as before. In this case an additional degree of freedom was lost due to totalling (6). The results are to be found in Fig. 3. The P value for the Goodness of Fit test was 0.13. Accordingly, the estimate of the population in any suspension was based on the average of counts made on five fields from each of four slides. Such estimates were made on 96 different suspensions. These are presented in Table V.

Immediately after making the direct counts, each suspension was reshaken and plated in quadruplicate at 0.001 and 0.0002 dilutions on Czapek's agar. Incubation was at 25° C. for seven days.

In order to determine the effect of soil in the medium, a second set of plates was prepared from the original suspension. In this case, the procedure was the same as for the previous set except that a plate at the 0.001 dilution contained 0.1 gm. of soil and one at the 0.0002 dilution contained 0.02 gm. This was accomplished by sterilizing 10 gm. of soil in sufficient water to make the 90 ml. blank used for preparing the 0.001 dilution. Twelve different soils were used in this study.

The results of the two plating experiments are shown in Table V.



EXPECTED NUMBER PER CLASS

1 1 3 5 10 10 20 20 10 10 5 3 1 1

OBSERVED NUMBER PER CLASS

0 0 2 7 16 14 26 20 5 8 2 0 0 0

CLASS BOUNDARIES

.3 .43 .71 1.06 1.65 2.19 3.36 4.88 5.99 7.78 9.49 11.67 13.28

FINAL X^2 = 19.83 n = 13 P = .09

Fig. 2. X^2 distribution on 100 slides for direct counts of spores of a saltant of *H. sativum* P.K.& B.

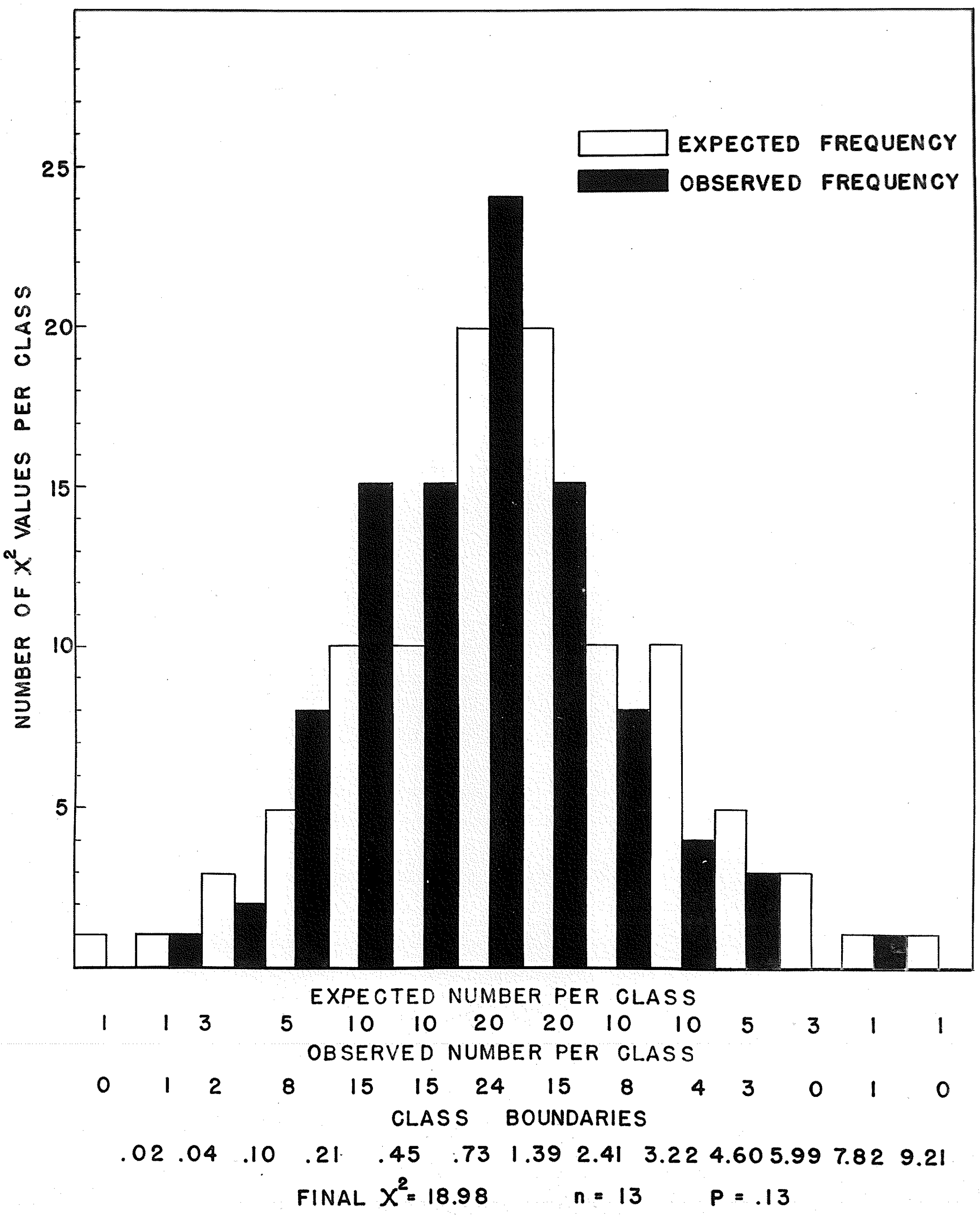


Fig. 3. X^2 distribution on 96 suspensions for direct counts of spores of a saltant of H. sativum P.K.& B.

Table IV. Microscopic counts on a saltant of *H. sativum* suspended in water -- 5 fields from each of 4 slides.

Suspensions	spores				spores plus mycelium			
1.	173	143	153	161	256	206	231	250
	148	166	147	154	237	237	219	221
	179	159	171	148	261	231	258	226
	188	157	164	166	315	222	253	258
	160	173	158	153	244	248	234	239
2.	178	148	159	159	254	241	254	255
	189	151	163	157	277	269	251	249
	165	173	177	163	245	273	266	258
	170	157	168	172	249	248	265	262
	145	155	154	160	231	224	243	256
3.	6	10	9	8	27	28	26	21
	10	9	9	12	29	24	21	27
	12	10	15	11	26	29	34	29
	11	16	11	12	32	33	29	31
	9	14	13	14	31	30	29	36
4.	6	8	9	10	21	23	29	25
	9	12	15	13	28	27	38	33
	9	8	10	9	25	24	27	26
	4	9	9	8	23	24	30	21
	8	6	7	9	28	25	28	24

Table IV. Cont.

Suspensions	spores				spores plus mycelium			
5.	7	11	10	9	25	28	27	24
	14	7	15	14	32	24	33	36
	13	8	12	12	33	26	29	31
	10	12	11	10	29	32	32	28
	12	9	8	13	33	26	27	30
6.	16	15	12	12	33	38	29	28
	12	11	10	14	25	29	28	21
	16	17	18	11	33	43	30	25
	13	16	11	16	31	39	28	37
	12	18	15	15	30	35	32	38
7.	35	34	40	40	61	56	70	66
	26	28	30	38	63	50	63	65
	36	26	35	30	60	53	69	58
	34	36	38	43	63	51	67	63
	40	33	44	39	44	54	71	65
8.	8	10	10	11	23	27	35	36
	7	6	15	9	21	22	36	39
	11	9	7	8	33	20	38	29
	11	4	9	12	34	18	36	34
	8	12	10	10	33	33	32	32

Table IV. Cont.

Suspensions	spores				spores plus mycelium			
9.	106	111	108	124	160	148	159	168
	143	133	123	104	174	175	176	151
	115	110	126	141	156	160	157	173
	112	109	103	118	145	151	149	149
	116	121	109	117	161	166	183	152
10.	58	53	54	63	115	94	84	101
	61	60	56	55	117	97	96	93
	78	56	60	61	111	97	101	90
	65	71	44	45	120	105	85	89
	66	62	63	56	105	102	93	96
11.	105	119	121	110	143	157	169	163
	142	123	137	124	177	164	176	168
	114	131	126	107	159	172	183	159
	113	132	117	120	159	198	160	162
	119	107	123	134	166	141	165	171
12.	80	98	91	94	112	124	132	127
	99	94	101	81	139	131	146	119
	108	88	99	89	143	112	140	125
	94	102	92	106	135	139	128	138
	100	95	105	102	146	128	142	145

Table IV. Cont.

Suspensions	spores				spores plus mycelium			
13.	17	11	12	15	31	23	26	30
	10	8	17	12	27	24	35	26
	5	13	10	14	25	27	26	26
	14	10	11	13	23	29	27	25
	16	16	19	11	26	32	36	22
14.	31	31	28	27	54	54	51	59
	43	24	29	32	69	52	54	50
	24	33	26	34	65	68	51	55
	30	26	33	24	64	57	60	61
	32	30	31	30	63	55	57	58
15.	9	5	9	12	23	18	21	26
	7	10	10	9	19	21	20	24
	9	8	7	10	24	22	22	26
	10	9	8	9	28	22	18	28
	12	6	9	7	33	15	19	21
16.	6	11	9	6	17	25	21	25
	7	6	10	9	12	28	26	27
	9	10	12	13	16	33	27	23
	7	8	8	11	17	29	26	27
	6	12	7	9	16	32	19	31

Table IV. Cont.

Suspensions	spores				spores plus mycelium			
17.	5	5	4	6	13	11	15	14
	4	4	6	0	10	8	12	12
	4	7	4	6	12	13	10	13
	1	5	4	7	10	14	16	13
	7	3	2	7	12	11	10	16
18.	11	10	12	13	29	25	23	25
	15	15	17	10	28	22	27	24
	8	10	8	11	21	26	26	23
	11	5	10	10	24	26	24	20
	12	9	12	14	25	24	23	27
19.	7	3	6	4	11	10	13	9
	0	7	4	7	9	14	10	13
	5	3	0	4	10	11	9	13
	6	6	4	3	17	10	10	9
	6	3	6	4	13	15	10	12
20.	7	10	9	14	17	19	19	25
	9	10	11	16	18	17	20	28
	7	8	8	7	12	19	17	17
	10	17	6	11	23	29	16	24
	11	13	7	9	20	26	15	18

Table IV. Cont.

Suspensions	spores				spores plus mycelium			
21.	2	3	4	4	11	9	13	17
	6	5	5	0	15	10	11	12
	4	2	5	4	11	11	14	14
	4	5	1	8	14	15	10	13
	6	6	7	6	14	15	17	13
22.	19	14	11	12	21	35	25	25
	16	18	14	14	31	36	26	32
	13	20	10	18	29	38	26	36
	17	17	12	21	38	40	25	40
	13	17	16	14	28	36	31	37
23.	4	10	8	4	11	23	17	17
	6	8	12	5	15	22	22	16
	7	7	7	6	14	22	13	15
	4	8	5	9	16	17	18	17
	8	9	4	8	13	21	15	18
24.	10	9	14	9	25	27	30	22
	16	12	12	11	29	28	25	25
	17	11	12	12	29	22	23	28
	9	15	12	17	30	32	27	30
	14	12	16	14	29	30	26	29

Table IV. Cont.

Suspensions	spores				spores plus mycelium			
25.	16	19	26	19	32	32	44	34
	18	25	22	21	33	42	45	43
	19	18	17	26	25	31	34	41
	21	24	26	26	39	40	43	43
	20	24	27	23	42	46	45	49
26.	2	6	5	5	15	15	14	12
	5	4	5	7	14	13	13	14
	7	4	6	4	13	11	15	13
	7	9	9	4	16	17	18	14
	7	7	5	9	17	17	15	21
27.	4	5	4	4	12	15	12	11
	6	6	6	8	14	13	14	19
	5	4	7	7	14	10	16	15
	4	7	5	4	11	16	13	12
	3	6	6	6	14	13	16	16
28.	11	14	17	15	30	34	37	37
	10	18	21	16	28	35	37	39
	14	17	15	18	32	38	39	40
	19	23	20	17	38	47	36	40
	20	19	18	21	39	36	34	42

Table IV. Cont.

Suspensions	spores				spores plus mycelium			
29.	24	27	29	25	40	46	48	41
	23	28	26	33	43	46	44	56
	28	29	31	27	47	57	55	41
	26	32	28	26	52	58	49	38
	21	27	30	29	39	51	51	39
30.	7	6	3	6	15	15	13	14
	5	4	5	2	12	12	14	18
	5	5	8	11	16	17	14	17
	6	5	5	7	18	18	10	16
	5	9	6	4	14	19	16	19
31.	6	5	5	9	14	13	11	21
	5	3	4	4	11	9	13	16
	9	5	4	4	14	14	12	14
	5	8	7	8	13	18	19	13
	1	4	5	5	11	10	11	13
32.	9	0	6	5	15	12	13	12
	5	7	8	5	13	17	13	15
	6	7	2	10	11	16	10	18
	8	10	6	6	18	13	14	15
	6	6	7	7	14	14	14	14

Table IV. Cont.

Suspensions	spores				spores plus mycelium			
33.	8	9	12	9	21	24	27	24
	8	8	12	8	24	17	24	17
	10	6	10	12	23	26	32	26
	8	9	15	9	20	23	29	25
	10	12	6	5	27	26	26	23
34.	57	48	65	67	87	79	108	102
	53	56	72	71	77	95	103	114
	56	59	70	64	68	81	105	109
	51	63	64	63	75	96	106	95
	67	59	68	69	86	93	114	98
35.	49	54	46	53	73	74	74	88
	57	63	46	50	72	86	73	78
	60	56	49	67	91	84	76	93
	52	62	50	56	86	89	81	87
	55	45	57	73	89	70	84	111
36.	6	4	4	3	15	10	12	12
	6	5	4	4	17	14	11	13
	5	4	4	3	12	11	11	10
	4	6	9	6	9	13	14	14
	6	7	2	6	14	15	15	12

Table IV. Cont.

Suspensions	spores				spores plus mycelium			
37.	10	11	12	14	27	33	33	41
	15	12	14	11	38	29	37	36
	13	10	9	14	37	31	28	37
	9	13	13	12	29	35	35	35
	12	13	11	11	34	29	33	34
38.	5	4	1	4	14	9	6	10
	0	3	5	1	12	8	11	7
	7	1	5	5	9	11	13	9
	4	6	2	3	14	13	10	9
	4	3	6	5	13	8	8	14
39.	6	7	8	10	13	16	18	19
	10	6	7	6	21	16	23	15
	9	7	5	7	15	17	14	16
	7	9	7	5	15	19	16	16
	7	5	8	8	16	20	14	19
40.	7	6	9	9	18	18	19	18
	10	7	8	11	21	16	19	23
	8	5	6	5	19	13	15	17
	10	7	6	8	24	16	16	19
	9	4	8	7	24	15	17	16

Table IV. Cont.

Suspensions	spores				spores plus mycelium			
41.	28	21	26	23	65	44	44	49
	24	23	20	21	52	49	43	42
	22	24	22	25	50	49	42	47
	30	24	23	24	60	43	37	41
	20	26	24	22	59	48	45	38
42.	7	3	5	6	18	12	10	17
	3	6	6	2	16	15	14	15
	9	9	11	8	17	16	20	15
	8	6	5	5	17	14	14	12
	6	6	2	9	18	18	16	21
43.	46	42	53	51	78	79	78	84
	43	45	58	55	89	82	91	89
	48	44	43	50	81	76	88	82
	55	52	47	45	92	88	84	83
	46	51	49	42	87	84	83	80
44.	7	6	6	5	15	12	12	12
	6	5	4	6	13	9	10	11
	7	5	6	5	14	11	14	9
	4	5	5	5	10	13	10	11
	7	7	6	7	15	15	13	14

Table IV. Cont.

Suspensions	spores				spores plus mycelium			
45.	7	8	8	7	20	19	17	19
	7	9	8	9	19	20	19	21
	11	7	9	8	21	18	20	16
	9	6	8	11	23	19	21	24
	10	11	10	9	21	24	23	22
46.	14	20	18	19	30	33	36	39
	17	24	19	17	33	45	38	39
	17	19	23	22	34	36	42	43
	19	19	19	21	39	35	37	40
	21	18	18	20	43	32	37	38
47.	7	6	6	7	17	14	13	14
	8	7	7	7	19	18	15	15
	9	9	8	7	22	20	14	14
	7	7	8	9	18	17	15	20
	7	6	8	8	17	16	16	19
48.	21	16	18	19	38	32	34	37
	17	18	22	19	36	37	39	35
	17	19	17	18	33	36	33	35
	18	20	19	17	39	41	37	33
	19	17	21	19	35	34	36	36

Table IV. Cont.

Suspensions	spores				spores plus mycelium			
49.	4	5	4	5	11	12	10	8
	3	3	3	5	12	9	10	11
	4	4	3	4	11	10	7	9
	5	4	5	3	14	11	12	9
	2	4	3	4	10	13	9	12
50.	11	14	14	13	27	27	25	23
	18	16	15	11	34	26	29	20
	15	17	14	18	29	31	28	37
	14	15	17	13	25	29	28	24
	13	20	12	12	25	33	22	24
51.	23	23	26	25	45	46	40	43
	21	21	23	21	46	47	46	38
	27	20	33	28	54	41	53	46
	30	26	32	23	51	48	54	44
	23	25	24	29	49	51	37	50
52.	8	5	7	7	14	11	14	16
	6	4	5	8	12	11	12	14
	4	4	6	6	12	13	14	15
	5	3	6	5	13	10	14	13
	6	4	5	4	12	14	11	10

Table IV. Cont.

Suspensions	spores				spores plus mycelium			
53.	10	10	14	14	28	27	28	26
	13	13	15	13	26	26	28	30
	11	12	16	14	23	29	26	27
	13	13	10	15	24	23	25	29
	10	10	10	13	25	22	21	27
54.	12	10	18	16	26	27	31	28
	15	15	16	19	30	34	30	34
	14	10	16	10	25	22	31	19
	12	15	17	14	25	32	33	23
	15	15	15	14	29	29	23	26
55.	46	34	41	39	68	67	66	65
	42	44	48	37	68	67	75	63
	39	41	36	49	69	65	62	71
	46	40	39	41	74	70	64	68
	56	48	54	31	79	73	78	64
56.	29	20	26	22	49	36	45	37
	27	18	28	31	42	32	49	49
	26	23	26	20	49	35	52	38
	21	22	25	23	42	40	43	46
	20	24	24	25	39	38	47	51

Table IV. Cont.

Suspensions	spores				spores plus mycelium			
57.	19	17	13	12	39	31	33	28
	17	13	15	18	42	34	33	45
	16	14	14	15	35	34	30	30
	14	17	14	12	33	40	29	31
	15	15	12	14	37	33	32	32
58.	28	31	27	29	48	55	44	51
	26	27	31	29	34	53	54	54
	29	30	25	27	50	55	47	46
	29	25	27	29	53	43	46	47
	30	28	23	26	52	49	40	44
59.	4	5	5	4	11	11	14	13
	5	9	7	6	13	19	15	14
	4	3	8	4	13	10	17	10
	4	5	5	5	14	13	12	12
	3	4	5	6	14	13	13	16
60.	4	3	2	3	12	10	7	8
	4	4	2	3	10	11	9	11
	3	3	3	4	10	10	12	11
	4	4	3	5	10	9	9	14
	0	5	4	3	9	9	10	9

Table IV. Cont.

Suspensions	spores				spores plus mycelium			
61.	5	5	5	4	16	20	19	9
	4	6	4	6	15	19	18	21
	4	4	2	5	18	15	14	21
	4	3	5	6	19	11	17	23
	5	4	3	3	19	16	12	17
62.	3	6	6	7	17	16	17	21
	5	4	4	4	16	16	13	18
	4	5	5	3	12	17	20	19
	3	0	5	4	13	10	18	16
	4	6	4	3	12	18	18	14
63.	3	2	3	4	12	9	10	12
	4	5	3	4	12	16	7	13
	4	4	4	3	14	12	14	10
	3	4	5	1	10	10	15	9
	2	4	4	3	8	13	12	11
64.	27	22	32	26	49	40	54	46
	29	24	28	29	53	43	47	51
	26	33	28	25	46	51	45	43
	31	29	24	30	54	52	42	54
	22	26	27	28	38	47	48	47

Table IV. Cont.

Suspensions	spores				spores plus mycelium			
65.	19	17	18	19	42	40	38	39
	18	18	22	20	44	37	46	44
	20	15	21	16	46	36	43	38
	16	19	15	16	39	45	38	36
	21	20	16	20	43	45	37	41
66.	10	11	8	9	28	29	24	24
	9	9	10	11	30	27	31	30
	10	10	13	11	29	30	32	28
	12	10	10	13	31	28	29	33
	11	12	10	10	31	32	27	28
67.	14	15	13	15	32	36	30	38
	13	13	10	15	36	35	28	40
	10	11	11	12	34	33	31	36
	16	15	15	12	38	39	37	34
	14	15	14	11	37	38	37	31
68.	10	9	7	6	28	21	22	17
	7	10	8	11	21	26	26	28
	6	7	8	6	19	18	23	18
	6	6	6	9	17	16	19	22
	8	7	9	10	20	19	24	24

Table IV. Cont.

Suspensions	spores				spores plus mycelium			
69.	3	2	2	3	9	7	7	11
	2	1	3	4	10	7	7	13
	2	3	1	3	7	10	6	10
	3	3	3	0	9	11	10	5
	2	4	2	2	8	12	8	7
70.	4	6	3	4	9	13	9	11
	3	4	5	2	7	10	14	7
	4	4	2	4	11	9	6	12
	3	3	4	8	8	10	13	17
	5	2	6	4	13	8	15	10
71.	2	4	3	2	7	9	9	6
	2	3	1	3	6	9	5	7
	4	0	2	0	6	5	6	6
	4	1	2	3	10	7	8	8
	2	2	2	4	9	7	7	11
72.	21	23	25	20	43	45	46	41
	30	22	25	25	53	40	50	38
	23	21	26	26	47	38	48	48
	27	26	23	28	51	49	47	51
	26	33	29	23	48	57	52	49

Table IV. Cont.

Suspensions	spores				spores plus mycelium			
73.	3	4	4	3	9	11	10	8
	3	4	1	3	8	10	5	9
	3	3	3	5	8	9	9	12
	4	3	4	3	10	8	12	9
	2	4	0	2	8	6	8	7
74.	4	2	2	2	9	10	8	10
	0	1	2	2	10	6	10	9
	2	5	6	1	7	9	14	10
	6	0	2	5	8	7	12	12
	3	3	2	3	9	10	8	7
75.	8	11	11	9	19	23	22	18
	11	10	7	10	24	25	20	23
	12	15	14	9	23	23	23	17
	16	10	12	13	26	20	24	26
	12	16	10	10	23	25	21	20
76.	11	9	10	11	29	24	26	27
	12	8	11	10	30	26	28	26
	13	14	13	9	33	25	32	24
	9	10	6	14	23	26	27	35
	10	10	16	11	27	29	30	30

Table IV. Cont.

Suspensions	spores				spores plus mycelium			
77.	21	23	27	33	49	40	52	58
	29	31	35	31	51	53	59	50
	33	34	31	36	57	60	55	61
	32	32	39	34	53	57	63	59
	30	33	30	33	50	58	51	56
78.	15	14	9	13	29	25	20	25
	15	18	14	18	30	36	26	32
	15	16	21	17	26	29	40	35
	9	17	13	16	26	32	26	30
	14	16	12	14	29	29	26	29
79.	14	28	19	20	34	45	38	40
	24	16	23	21	46	32	43	39
	23	22	23	18	40	41	40	39
	23	26	16	14	42	39	37	38
	18	27	18	19	36	38	39	41
80.	4	4	5	4	10	9	9	9
	3	4	0	6	8	10	11	12
	3	6	3	3	9	10	8	9
	5	6	4	3	12	11	8	7
	4	6	3	5	11	12	9	12

Table IV. Cont.

Suspensions	spores				spores plus mycelium			
81.	3	2	4	2	13	12	15	14
	5	2	2	2	16	14	15	18
	2	2	1	4	13	11	10	17
	2	3	3	2	15	15	11	10
	3	4	6	5	14	15	19	16
82.	1	1	1	2	8	9	11	12
	0	2	0	3	7	11	9	15
	2	0	2	2	10	9	9	10
	2	1	2	0	12	10	8	7
	0	3	1	4	10	14	11	10
83.	3	2	5	1	15	14	21	12
	2	3	3	2	12	18	14	14
	3	3	2	4	16	16	11	15
	4	3	3	3	14	18	19	15
	1	1	3	4	16	12	13	17
84.	7	10	8	9	11	14	11	12
	9	8	8	10	13	10	13	14
	6	7	6	8	9	9	10	9
	8	9	9	10	10	12	14	10
	8	11	8	9	9	16	11	10

Table IV. Cont.

Suspensions	spores				spores plus mycelium			
85.	15	11	15	16	34	26	35	36
	17	15	13	16	36	34	32	34
	19	18	20	19	29	39	43	40
	13	16	17	12	31	35	38	33
	10	20	17	18	34	42	34	39
86.	3	2	2	3	10	9	8	11
	4	0	3	3	13	6	10	10
	3	4	4	2	10	15	11	8
	2	3	1	4	11	12	8	14
	3	3	2	2	12	11	10	11
87.	7	6	5	6	20	13	16	14
	4	5	5	6	12	14	14	17
	5	4	4	5	12	12	13	14
	4	4	7	3	11	13	21	10
	5	4	4	9	15	15	15	23
88.	7	6	8	10	15	15	19	23
	7	5	6	10	14	15	14	24
	5	7	6	6	13	16	16	15
	6	7	7	8	14	14	18	19
	7	6	8	7	16	15	18	15

Table IV. Cont.

Suspensions	spores				spores plus mycelium			
89.	10	6	6	5	18	15	16	13
	8	4	5	7	16	10	16	17
	6	6	6	6	16	17	17	13
	7	7	7	9	15	16	18	19
	8	9	8	6	17	20	15	17
90.	3	4	3	6	12	11	13	17
	4	2	3	3	14	9	15	11
	4	4	3	6	13	10	12	14
	3	4	5	2	11	12	14	13
	3	4	7	1	12	14	16	14
91.	7	4	4	4	19	14	10	13
	4	5	6	6	18	14	14	15
	4	4	5	6	17	12	16	14
	6	5	5	6	15	13	14	18
	5	4	7	4	12	13	17	9
92.	3	4	4	3	15	12	14	12
	2	3	2	1	12	12	8	8
	4	3	0	3	18	10	7	12
	3	4	6	6	15	16	15	18
	7	5	3	6	21	18	12	16

Table IV. Cont.

Suspensions	spores				spores plus mycelium			
93.	4	6	7	9	15	19	16	20
	7	3	2	5	17	11	9	16
	10	6	6	6	19	15	16	13
	3	3	6	5	9	8	14	13
	8	11	8	7	21	23	15	17
94.	4	6	5	6	13	14	16	18
	4	5	4	7	11	16	13	18
	5	5	7	6	15	14	19	12
	5	4	7	8	17	13	17	19
	7	6	5	4	20	13	16	10
95.	12	11	12	10	33	27	31	23
	13	11	6	9	29	29	20	21
	8	9	10	8	22	20	24	18
	12	10	9	11	26	24	20	29
	10	12	9	13	22	28	19	32
96.	1	4	2	6	9	12	13	14
	3	1	2	1	13	11	12	7
	2	1	3	3	9	10	15	10
	2	3	5	0	11	13	16	7
	5	2	1	0	15	9	9	8

Table V. Estimates on a saltant of H. *sativum* made by four methods, - I. direct count of spores, II. direct count of spores plus fragments of mycelium, III. plate counts, and IV. plate counts with soil in medium. (X 1000)

		Method			
Soil	Susp'ns	I	II	III	IV
a.	1.	1610.0	2423.0	707.5	467.5
	2.	1630.0	2535.0	913.0	487.0
	3.	110.0	286.0	87.5	67.5
	4.	86.0	264.0	57.5	40.0
	5.	106.0	292.0	66.0	25.0
	6.	140.0	315.0	106.0	60.0
	7.	351.0	606.0	211.0	130.0
	8.	93.0	305.0	111.0	62.5
b.	9.	1124.0	1591.0	412.5	307.5
	10.	593.0	995.0	172.5	110.0
	11.	1212.0	1656.0	522.5	452.5
	12.	959.0	1325.0	355.0	261.0
	13.	127.0	273.0	80.0	65.0
	14.	299.0	563.0	115.0	90.0
	15.	87.0	225.0	55.0	45.0
	16.	88.0	238.0	75.0	45.5

Table V. Cont.

		Methods			
Soil	Susp'ns	I	II	III	IV
c.	17.	45.0	122.0	31.25	25.0
	18.	111.0	246.0	53.75	35.0
	19.	43.0	114.0	25.0	15.0
	20.	110.0	199.0	56.25	40.0
	21.	43.0	105.0	40.75	32.5
	22.	153.0	317.0	71.25	52.5
	23.	69.0	171.0	37.75	30.0
	24.	127.0	273.0	55.0	42.5
d.	25.	218.0	391.0	112.0	65.0
	26.	58.0	148.0	93.5	45.0
	27.	53.0	138.0	46.0	25.0
	28.	171.0	369.0	126.0	90.0
	29.	274.0	470.0	106.0	70.0
	30.	57.0	153.0	45.0	26.0
	31.	53.0	135.0	31.25	20.0
	32.	63.0	140.0	26.0	22.0

Table V. Cont.

		Methods			
Soil	Susp'ns	I	II	III	IV
e.	33.	93.0	242.0	53.5	40.0
	34.	621.0	945.0	397.5	250.0
	35.	550.0	829.0	387.5	267.5
	36.	49.0	127.0	31.0	27.5
	37.	119.0	335.0	40.0	32.0
	38.	37.0	416.0	2.0	1.0
	39.	74.0	169.0	46.0	35.0
	40.	75.0	181.0	37.5	25.0
f.	41.	235.0	473.0	240.0	157.5
	42.	61.0	158.0	60.0	35.0
	43.	486.0	839.0	265.0	170.0
	44.	57.0	121.0	62.5	36.5
	45.	86.0	206.0	72.5	47.5
	46.	192.0	374.0	171.0	110.0
	47.	74.0	166.0	87.5	50.0
	48.	185.0	358.0	150.0	102.5

Table V. Cont.

Soil	Susp'ns	Methods I	II	III	IV
g.	49.	38.0	105.0	31.0	23.5
	50.	146.0	273.0	135.0	90.0
	51.	251.0	464.0	195.0	102.5
	52.	54.0	127.0	38.5	21.0
	53.	124.0	255.0	100.0	62.5
	54.	144.0	278.0	120.0	77.5
	55.	425.0	688.0	288.5	162.5
	56.	240.0	439.0	170.0	105.0
h.	57.	140.0	340.0	70.0	35.5
	58.	275.0	482.0	160.5	110.0
	59.	50.0	133.0	40.0	25.5
	60.	33.0	100.0	17.5	8.0
	61.	43.0	169.0	32.5	17.0
	62.	42.0	160.0	30.0	19.5
	63.	34.0	114.0	27.0	16.0
	64.	273.0	475.0	220.0	122.5

Table V. Cont.

		Methods			
Soil	Susp'ns	I	II	III	IV
i.	65.	183.0	408.0	103.0	63.5
	66.	104.0	290.0	69.0	41.0
	67.	132.0	350.0	95.5	56.0
	68.	78.0	214.0	56.0	32.5
	69.	24.0	87.0	18.5	10.0
	70.	40.0	106.0	32.5	20.0
	71.	23.0	74.0	18.0	9.5
	72.	251.0	470.0	100.0	55.0
j.	73.	31.0	88.0	20.5	12.0
	74.	26.0	92.0	18.0	11.5
	75.	113.0	222.0	94.0	53.0
	76.	108.0	278.0	93.5	53.0
	77.	313.0	546.0	207.5	121.0
	78.	148.0	290.0	72.0	43.5
	79.	211.0	393.0	165.5	97.5
	80.	40.0	98.0	29.5	13.5

Table V. Cont.

Soil	Susp'ns	Methods I	II	III	IV
k.	81.	29.0	141.0	25.0	19.5
	82.	12.0	101.0	25.5	12.5
	83.	27.0	151.0	24.5	17.0
	84.	79.0	113.0	77.5	45.0
	85.	158.0	352.0	102.5	42.5
	86.	29.0	104.0	22.5	15.0
	87.	51.0	147.0	57.5	37.5
	88.	69.0	139.0	55.0	35.0
l.	89.	68.0	162.0	40.0	25.0
	90.	37.0	128.0	22.5	17.5
	91.	50.0	143.0	40.0	35.0
	92.	36.0	135.0	35.0	20.0
	93.	61.0	153.0	38.75	30.0
	94.	55.0	152.0	37.5	30.0
	95.	102.0	248.0	46.25	37.5
	96.	23.0	111.0	21.25	15.0

Since estimates of spores and of spores and fragments of mycelium were based on counts on the same fields, it is obvious that the latter would be larger. Estimates made from spores and fragments were not considered further in this study.

Estimates made by the plate method (Method III) were smaller than those made by the direct count method in 92 of the 96 suspensions studied. The difference between means of estimates made by the two methods was 80.34.* The necessary difference for significance at the 0.01 per cent level was found to be 44.32 by a formula adapted from information found in Goulden's text (10).

The calculations follow:

$$\text{Nec. Diff.} = \sqrt{\frac{2\ (\text{Error Mean Square})}{\text{No. of replicates}}} \times t_{.01} \text{ for D.F. for Error.}$$

$$\text{or} = \sqrt{\frac{2\ (13{,}788.02)}{96}} \times 2.63$$

$$= 44.32$$

Estimates made by Method IV, in which case particles of sterile soil were present in the culture medium, were smaller than those made by Method III in all of the 96 suspensions tested. The data on these two methods were submitted to an analysis of variance. This follows:

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*The above calculations were carried out on coded data. Actual difference between estimates was 80,340 per ml.

Source	D.F.	M.S.	F.	F.05
Soils	11	63,332.27	50.32	1.90
Treatments x soils	12	9,131.34	7.25	1.87
Replicates x soils	84	22,243.03	17.67	1.87
Error	84	1,258.66		

This was accepted as evidence that some factor or factors associated with particles of sterile soil in the medium affected the growth of the saltant, and that this effect was different in different soils. The significant effect shown for replicates in the analysis was expected since it was not feasible to prepare suspensions with the same number of spores, particularly when the suspensions were made on different days and from different cultures.

It is conceivable that sterile soil in the medium could change the pH of the medium appreciably and be a factor responsible for the lower estimates reported for Method IV. This would differ with soils of different pH. The effect of small amounts of soil in the medium on pH was tested by the following procedure. A pH determination was made on duplicate 10 ml. quantities of Czapek's medium, Czapek's medium plus 0.1 gm. soil, and Czapek's medium plus 0.02 gm. soil. A Coleman pH electrometer was used. The experiment was replicated on the 12 soils used in this study. The results are presented in Table Vl. It should be noted that these quantities of soil in the medium are 100 times as great as would be present in plates prepared from soil at the 0.001 and 0.0002 dilutions. The effect of slight differences in pH of Czapek's medium on numbers of this saltant developing in this medium were not determined in this study.

Table Vl. Effect of sterile soil in medium on pH.

		Soil per plate	
Soil	Check	0.1 gm.	0.02 gm.
a.	4.50	5.10 5.10	4.80 4.70
b.		5.50 5.50	4.90 4.95
c.		5.00 4.95	4.80 4.80
d.		6.90 7.00	5.60 5.60
e.		5.00 5.00	4.70 4.75
f.		5.10 5.05	4.80 4.80
g.		5.10 5.00	4.65 4.60
h.		4.95 4.90	4.75 4.75
i.		5.00 5.00	4.70 4.65
j.		4.80 4.85	4.70 4.70
k.		5.20 5.15	4.85 4.90
l.		5.00 4.90	4.75 4.80

DISCUSSION

The reason for the difference between estimates obtained by the direct spore count and by the plating method is not readily apparent. Several theories are advanced, however, which may account for this difference.

In the first instance, although data concerning percentage germination of this saltant are not available, various other strains of H. sativum P.K. & B., as reported by Christensen (4), showed germination ranging from 38 to 62 per cent in distilled water. It would scarcely seem likely that this strain would show 100 per cent germination. Consequently, the direct spore count would have a disadvantage in that all the spores counted might not be viable. This disadvantage would seem particularly evident in the case of suspension No. 38. At the time of counting, a note was made to the effect that the spores in this suspension were distorted and seemed smaller than those usually encountered. It was not surprising, therefore, that the estimate made from the plate count on this suspension was very low. In the second instance, although the suspensions were shaken before plating, the possibility remains that some spores were grouped together and that each group would give rise to only one growth on the plate. This would lead to an underestimation of the population as represented by the plate count. Thirdly, there is a possibility that oxygen tension was not optimum for germination throughout the medium. Information was not available concerning the oxygen requirements for germination of spores of this strain. However, various investigators (4)(19) have shown that most fungal spores germinate more readily on the surface of the medium.

As well, the reason for the difference in estimates made by the two plating methods is not readily explained. The following is presented in an attempt to account for this difference.

Christensen (4) has shown that various soil extracts added to distilled water reduced the germination of spores of his strains of H. sativum P.K. & B. by approximately 21 per cent. He reported further that boiling these extracts seemed to destroy the toxic principle. In this investigation, however, the soil dilution was subjected to sterilization temperatures and the effect was still evident. Further, it is possible that spores adhered to particles of soil. It is likewise possible that many of these particles settled to the bottom of the medium before it solidified. In this area conditions for germination may not have been optimum. Either of these phenomena conceivably could account for part of the reduction shown in the medium containing soil.

SUMMARY

(1) Estimates of population were made on 96 suspensions of a white fertile saltant of *Helminthosporium sativum* P.K. & B. by the direct microscopic method and by two plating methods.

(2) The validity of an estimate based on five fields from each of four slides per suspension was demonstrated.

(3) The difference between means of estimates made by (a) the direct spore count method and (b) the plate count method was shown to be significant.

(4) Estimates based on counts on agar plates containing sterile soil were lower than those on counts from plates without soil.

(5) Small quantities of sterile soil added to the medium raised the pH slightly. This increase was not the same for all soils.

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