Studies in the Genera Cytosporina, Phomopsis, and Diaporthe.

III. On the Pathogenicity of Cytosporina ludibunda and its Saltants.

BY

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With twelve Figures in the Text.

CONTENTS.

I. INTRODUCTION.

IN the first (14) and second (7) papers of this series an account was given of the occurrence of saltation in some of the genera under investigation. The main characteristics of the saltants, as observed in standard synthetic medium, as well as variations in general morphological characters with change of medium were described. Finally an attempt was made to compare the saltants with certain authentic species of *Phomopsis* and *Diaporthe*. It is proposed now to deal with the attacking power on apple of the strains under investigation, but, for the sake of convenience, only the saltants of *Cytosporina ludibunda* will be considered in the present paper, comparison with *Phomopsis* and *Diaporthe* being left for a later communication.

As mentioned in the preceding paper (7), *Cytosporina ludibunda* from 1920 onwards has been utilized for the work on fungal invasion carried out in this laboratory. In 1926, the year in which saltation was first observed by the writer, Horne ((13) p. 98, Fig. 44), using the parent strain of *Cytosporina ludibunda*, observed certain irregularities in the curves representing

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progress of invasion with age of Bramley's Seedling apples. But in the following year, when MK, a saltant of *C. ludibunda*, was used instead of the parent strain, the results were more regular ((12), p. 126, Fig. 47 ; (13) p. 99, Fig. 45). It was, therefore, thought that the irregularities observed in 1926 were possibly due wholly or in part to dissociation of the parent strain of *C. ludibunda* into saltants of varying attacking power, within the apple tissue.

The present work was therefore undertaken to test the attacking power of the parent and the more stable saltants of *C. ludibunda*, using Bramley's and Worcester apples as hosts. The experiments were carried out for three successive years (1927–9). A list of the strains used is given below, together with reference to the earlier paper (7), where their origin is described.

C Parent strain of *C. ludibunda* ((7) p. 351).

CC Brown saltant with large dark-brown stromata—derived from C ((7) pp. 351–5).

CC_2 Pale orange-yellow non-sporing saltant—derived from CC via CC_1 ((7) p. 353).

CA_1 Grey saltant with few stromata—derived from C through CA ((7) p. 352).

CA_2 Grey non-sporing saltant derived from CA_1 ((7) p. 352).

CA_3 Grey saltant forming numerous stromata derived from CA_1 ((7) p. 353).

CA_4 Black non-sporing saltant derived from CA_3 ((7) p. 353).

MK As mentioned in the previous paper several monohyphal cultures were obtained from the parent strain. Of these, certain cultures which showed a strong resemblance were classed together as MK. This particular group was not studied in detail. Cultures of MK were used for inoculation purposes for a time by Horne and then abandoned in favour of CA_2 (= CE). In standard medium culture MK develops a thin white mycelium, white substratum, and wide zonation. The stromata are large and dark brown and occur sporadically.

For summary of characters of the other strains observed in standard medium cultures see (7) p. 360, Table III.

The estimates of attacking power given in this paper are based on data of the rate of radial advance in apple fruit, calculated by the method formulated by Gregory and Horne (10). The data have been treated, as far as possible, by the method of statistical analysis (9). In experiments where different samples of apples were used for comparisons of attacking power without replicates, differences are regarded as significant only where the odds against the results being fortuitous exceed 100 : 1.

II. Pathogenicity of the Strains of *Cytosporina ludibunda*.

In 1927 two varieties of apple, Bramley's Seedling (from Canterbury) and Worcester Pearmain (from Exning), were used for the inoculation experiment. Four sets in all were available, viz. Early Bramley's, Late Bramley's, Early Worcester, and Late Worcester. Each set was subdivided into samples of twenty, and six strains (C, CC, CC_2, CA_3, CA_4, and MK) were tested. Each sample was inoculated with a given strain, individual apples being inoculated on opposite sides. The inoculated apples were stored at the Low Temperature Station, Cambridge, at 12° C. The dates of inoculation and estimation of different samples are given in tables showing the rate of advance of various strains. The period of storage for the inoculated apples was decided from knowledge of previous growth rate of *C. ludibunda* in apples at that temperature.

During storage a certain amount of loss through natural infection occurred among the inoculated Worcester, hence the number of apples used in each sample for determining radial advance was reduced from twenty to fourteen. The data of mean radial advance in cm. per day for these samples is given in Table I. Owing to the fact that the majority of the apples in samples inoculated with CA_2 and many of those inoculated with CA_4 were completely decayed when examined, the actual value of radial advance should be higher than that given in the table.

Table I.

Worcester Pearmain Apples—Mean Radial Advance in cm. per day. 12° C. 1927.

	Early.			Late.		
Strain.	Date of inoculation.	Date of estimation.	Radial advance.	Radial advance.	Date of inoculation.	Date of estimation.
CA_2	Sept. 14	Dec. 10	> 0·0450	> 0·0680	Oct. 12	Dec. 10
CA_4	,, 14	,, 17	> 0·0330	> 0·0460	,, 11	,, 12
MK	,, 16	,, 17	0·0187	0·0237	,, 12	,, 17
CC	,, 16	,, 17	0·0188	0·0229	,, 12	,, 19
C	,, 15	,, 17	0·0152	0·0209	,, 13	,, 12
CC_2	,, 15	,, 19	0·0000	0·0000	,, 12	,, 19

It is seen at once that the strains vary greatly in attacking power. They fall naturally into three groups: (i) Strong, CA_2 and CA_4, which are approximately three times as active as the parent strain (C); (ii) Intermediate, C, CC, and MK; and (iii) Weak, CC_2 alone, which proved quite inactive.

In the case of Bramley's Seedling, samples of inoculated apples were reduced to 18 individuals each owing to the loss by natural infection during storage. The data of mean radial advance in cm. per day for all the samples are given in Table II, where the strains are arranged in order of descending values of radial advance as observed for the early Bramley's.

TABLE II.

Bramley's Seedling Apples. Mean Radial Advance in cm. per day. 12° C. 1927.

	Early.			Late.		
Strain.	Inoculated.	Estimated.	Radial advance.	Radial advance.	Inoculated.	Estimated.
CA_2	Oct. 5	Dec. 10	0·0833	0·1100	Nov. 2	Dec. 10
CC	,, 7	,, 29	0·0109	0·0388	,, 3	,, 17
MK	,, 4	,, 13	0·0079	0·0116	Oct. 31	,, 7
C	,, 7	,, 13	0·0058	0·0060	Nov. 2	,, 7
CA_4	,, 5	,, 17	0·0051	0·0439	,, 1	,, 17
CC_2	,, 6	,, 17	0·0020	0·0016	,, 1	,, 19

Comparison of Tables I and II shows that the general grouping of the strains with respect to attacking power is affected to a certain extent by variety of apple, since CA_4 found in the 'strong' group in Worcester falls into the 'intermediate' group in the late Bramley's and into the 'weak' group in the early Bramley's.

It should also be noted that the order of strains is not identical in the two sets of Bramley's (early and late), but this is solely due to CA_4 which occupies the fifth and second place in early and late Bramley's respectively.

The significance of the difference between strain and strain as indicated in Table II has been tested, using the standard error of difference of means calculated for each set separately.

The S.E. for all the samples of the early Bramley's set is 0·0026. The S.E. of difference of any two means is therefore $0{\cdot}0026 \times \sqrt{2} = 0{\cdot}0037$. Similarly, the S.E. for all the samples of the late Bramley's is 0·0065. The S.E. of difference of any two means is 0·0092. Any difference of means which exceeds three times the S.E. of difference (0·0275) is regarded as significant for this set.

The difference between any two means can be readily ascertained from Table III, where in early Bramley's, for example, the difference between the means of C and CC is 0·0051, MK and CC_2 0·0069, and so on.

The degree of significance indicated by these figures is shown in diagrammatic form in Figs. 1 and 2 for early and late Bramley's respectively.

The following points will be noted in the diagrams: (1) CA_2 differs significantly from all the other strains in both sets of Bramley's. (2) In early Bramley's the remaining strains do not differ significantly. (3) In late Bramley's the strains differ significantly except the following pairs: CA_4 and CC; CC and MK; MK and C; MK and CC_2; C and CC_2.

When the strains are arranged in order of attacking power based on the significance of differences, the grouping differs in the early and late sets. In the early set the 'strong' group contains only a single strain CA_2.

The remaining strains fall into the 'weak' group, there being no intermediates. In the late set the strains again fall into three groups: strong CA_2; intermediate CA_4 and CC; weak C, CC_2, and MK.

TABLE III.

Differences between Mean Radial Advance, cm. per day, of Pairs of Strains. 1927.

Bramley's Seedling (Early).

Strain.	CA_2.	CC.	MK.	C.	CA_4.
CC	0·0724				
MK	0·0754	0·0030			
C	0·0775	0·0051	0·0021		
CA_4	0·0782	0·0058	0·0028	0·0007	
CC_2	0·0813	0·0089	0·0059	0·0038	0·0031

Bramley's Seedling (Late).

Strain.	CA_2.	CA_4.	CC.	MK.	C.
CA_4	0·0671				
CC	0·0722	0·0051			
MK	0·0994	0·0323	0·0272		
C	0·1050	0·0379	0·0328	0·0056	
CC_2	0·1094	0·0423	0·0372	0·0100	0·0044

The strains comprising the 'weak' group include, among others, C the parent strain and the saltant MK. These two do not differ significantly from each other in the experiments described above, although the actual figure for MK is in every case higher than that of C.

Certain supplementary experiments were made to determine whether a real difference between C and MK could be established. In these experiments the strains were tested on the same apples. Each apple was inoculated at four points with C and MK at alternate points. A further experiment was made in a similar way, in which MK (stock culture) was tested against MK, re-isolated from an apple previously inoculated with MK. The experiments carried out are as follows:

Experiment I. Twelve Bramley's Seedling apples inoculated with C and MK on December 8, 1927; kept 12 days. 20° C.

Experiment II. Thirteen Worcester Pearmain apples inoculated with C and MK on November 25, 1927; kept 11 days. 20° C.

Experiment III. Twelve Bramley's Seedling apples inoculated with MK (stock) and MK (re-isolated) on December 8, 1927; kept 12 days. 20° C.

From the data obtained for each experiment the mean radial advance for each strain was calculated. The significance of the difference of these

means has been tested by the method of t ((9) pp. 104–6). The values of mean radial advance, the values of t, and the probability (P) indicated by the latter are given in Table IV.

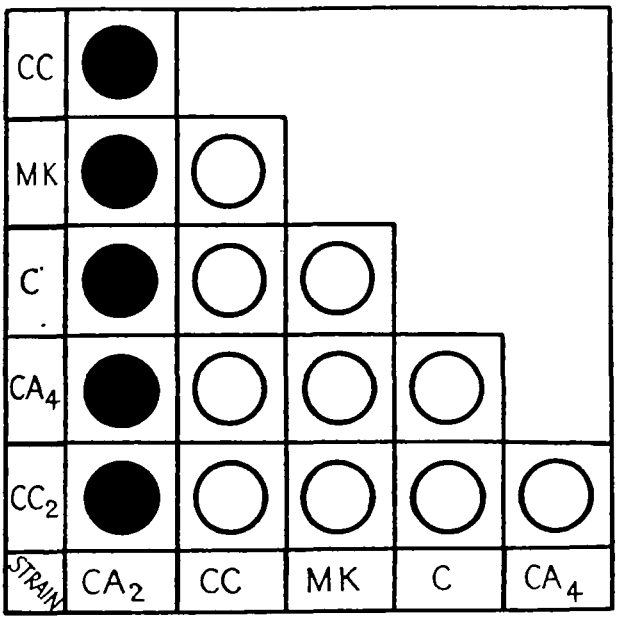

FIG. 1. Diagram showing degree of significance of difference between pairs of strains in early Bramley's Seedling apple, 1927. S.E. = 0·0037. For meaning of signs see below.

○ = a difference not exceeding 3SE (not significant)

⊗ = a difference between 3SE and 6SE (significant)

◉ = a difference between 6SE and 9SE (significant)

● = a difference exceeding 9SE (significant)

Key to Figs. 1–5 and 10–12.

It will be seen from the results of experiments I and II that MK in each case is again more active than C.

Of these results, however, only the first is significant, since out of 100 such random samples only one will by chance give a value of t exceeding

+3·10 or less than −3·10. The second result is not significant since about 5 out of 100 samples will give a value of t greater than + 2·26 or less than −2·26. The results taken as a whole may indicate a real difference in

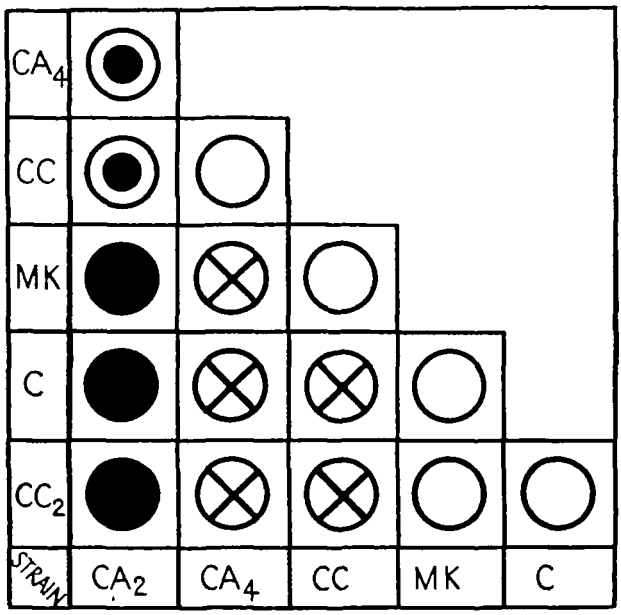

FIG. 2. Diagram showing degree of significance of difference between pairs of strains in late Bramley's Seedling apple, 1927. S.E. = 0·0092. For meaning of signs see p. 202.

attacking power between parent strain and MK. As for Experiment III, the difference in the mean values of the two MK cultures is negligible, indicating that the MK culture had not changed in attacking power whilst in apple tissue.

TABLE IV.

Attacking Power of MK and C and the Significance of their Difference.

Experiment.	Mean radial advance, cm. per day. MK	C	Value of t.	P.
I.	0·274	0·131	3·10	0·01
II.	0·383	0·265	2·26	0·05 – 0·02
	MK. (stock).	MK. (re-isolated).		
III.	0·056	0·058	0·17	0·9 – 0·8

In 1928 only one variety of apple, Bramley's Seedling (from Canterbury), was used. Two more strains (CA_1 and CA_3) than in the previous year were included in the test. The experimental method was modified. The

apples were divided into samples of 20 as before, but two strains instead of one were used for each sample, individual apples being inoculated with one strain on one side and with the other strain at a point opposite to the first. Successive samples contained one of the strains used for the preceding sample. Thus for the first sample, CA_4 and CA_2 were used, and for the second sample CA_2 and CA_3. The complete cycle was as follows:

CA_4 and CA_2; CA_2 and CA_3; CA_3 and CA_1; CA_1 and CC; CC and CC_2; CC_2 and C; C and MK; MK and CA_4.

The inoculations were completed between October 10 and October 14 (difference of four days) and the estimations between November 2 and November 28 (difference of twenty-six days). The longest time that elapsed between the estimation of the first and second samples inoculated with the same strain was fifteen days in the case of CA_4. The experiment was carried out at laboratory temperature (18° C.–20° C.).

The data of mean radial advance in cm. per day, obtained for each strain, on the basis of 18 half-apples in a sample is given in Table V.

TABLE V.

Mean Radial Advance in cm. per day (18° C–20° C). Bramley's Seedling Apple. 1928.

Strain.	Mean radial advance, cm. per day. Sample I.	Sample II.	Mean.
CA_2	0·0800	0·0642	0·0721
CA_1	0·0491	0·0655	0·0573
CA_3	0·0523	0·0419	0·0471
MK	0·0266	0·0396	0·0331
CC	0·0128	0·0254	0·0191
CA_4	0·0087	0·0274	0·0180
C	0·0148	0·0194	0·0171
CC_2	0·0028	0·0017	0·0023

It is seen from Table V that again the strains differ from one another, the differences between extremes being considerable (CC_2, 0·0028; CA_2, 0·0800). Further, the values from the first and second samples of any one strain are not the same. For example, CA_3 has a mean radial advance of 0·0523 cm. in the first and 0·0419 cm. in the second sample, and with CA_4 the mean radial advance is 0·0087 and 0·0274 cm. per day in the first and second samples respectively.

The significance of these observed differences among the strains has been tested by the analysis of variance. Owing to the fact that these strains have always been associated in pairs, there is a correlation between values of the radial advances of pairs of strains tested. By taking mean values of grouped figures obtained for replicates for each strain, the values obtained are not strictly speaking random values. To test whether this has introduced an appreciable error in the estimates of radial advance, the

variance represented by the differences between the grouped values has been calculated and compared with the remainder error. The figures have been further analysed for the significance of interaction, which in this case represents the variance of differences of the values of radial advance for each fungus as estimated on the two groups of 18 half-apples ; that is, where strain CA_2 is associated with CA_4 as against CA_2 associated with CA_3; the strain CC associated with CA_1 as against CC associated with CC_2, and so on. The full analysis is given in Table VI.

Since there are 16 samples, each consisting of 18 half-apples, there will be 287 degrees of freedom in all; made up of 7 degrees of freedom for strains; 1 for groups mentioned above; 7 for interaction, and 272 for error (variance within samples).

TABLE VI.

Analysis of Variance. Attacking Power of Strains on Bramley's Seedling Apple. 1928.

	Degrees of freedom.	Sum of squares.	Variance.	S.D.	Log S.D.	Value of z.
Strains	7	0·141520	0·02022	0·14200	−1·95192	+1·68744
Grouping	1	0·001591	0·00159	0·03988	−3·22186	+0·41750
Interaction	7	0·008072	0·00115	0·03391	−3·38404	+0·25532
Error (within sample)	272	0·187650	0·00069	0·02627	−3·63936	

For $n_1 = 7$ and $n_2 = 272$ 1 % point of value of z is about 0·5152.
„ $n_1 = 1$ „ $n_2 = 272$ 5 % „ „ „ 0·6729.
„ $n_1 = 7$ „ $n_2 = 272$ 5 % „ „ „ 0·3706.

The value of z for strains is +1·687. This value is more than three times the 1 per cent. point, indicating that, taken as a whole, the strains differ significantly.

The value of z for grouping is +0·4175, a value less than the 5 per cent. point, showing that the differences due to grouping is within the experimental error.

The value of z for interaction is +0·2553, which is also below the 5 per cent. point. Interaction, therefore, is not significant.

It has been shown by analysis of variance that the strains differ in attacking power. The precise nature of this difference will now be considered, using the average of the mean value of two samples (36 half-apples as given in the last column of Table V).

The standard deviation for the whole experiment is 0·02627. The standard error for groups of 36 half-apples is $\frac{0{\cdot}02627}{\sqrt{36}} = 0{\cdot}0044$. The standard error of difference between any two means is

$$(0{\cdot}0044 \times \sqrt{2}) = 0{\cdot}0062.$$

The difference between mean values of any given pair of strains is given in Table VII.

TABLE VII.

Differences between Mean Radial Advance (cm. per day) of Pairs of Strains. Bramley's Seedling Apple. 1928.

Strain.	CA_2	CA_1	CA_3	MK	CC	CA_4	C
CA_1	0·0148						
CA_3	0·0250	0·0102					
MK	0·0390	0·0242	0·0140				
CC	0·0530	0·0382	0·0280	0·0140			
CA_4	0·0544	0·0396	0·0294	0·0154	0·0014		
C	0·0550	0·0402	0·0300	0·0160	0·0020	0·0006	
CC_2	0·0698	0·0550	0·0448	0·0308	0·0168	0·0154	0·0148

The degree of significance indicated by the above figures is represented diagrammatically in Fig. 3.

It will be seen from the diagram that CA_2 differs significantly from all but CA_1; CA_3 from all but CA_1 and MK; CA_1 from all but CA_2 and CA_3; MK differs significantly from CA_2, CA_1, and CC_2 only. The strains CC, CA_4, C, and CC_2 do not differ from each other.

It has been shown by the analysis of variance that the differences in radial advance due to grouping are not significant. It does not necessarily follow that differences between every pair of values for individual strains should be without significance. It will be seen from Table VIII that the radial advance of CA_4 in sample II is about three times as great as that shown in sample I. In order to test whether any significance should be attached to this difference a further analysis was made. In this analysis the effect of samples on radial advance was determined for individual strains by calculating the standard error of difference of means $\left(\frac{S\sqrt{2}}{\sqrt{n}}\right)$ for each pair of values.

The result of this analysis is given in Table VIII, where the differences of mean values of radial advance of the two samples are shown for each strain in the fourth column. The fifth column gives the S.E. of difference for the samples for purposes of comparison with column 4.

It is seen that for CA_4 the difference between the mean values of the two samples is more than five times its standard error of difference, and for CC it is three times as great. If three times the S.E. is taken as the minimum for significance, the samples in their reaction to CA_4 can be regarded as significantly different, while for CC and MK, where the difference is almost three times S.E., as also for the remaining strains, the differences due to sampling are not significant.

The difference between samples of apples as brought out by their

behaviour towards CA_4 is probably an effect due to age of apples, since sample II with CA_4 was inoculated later than sample I and was kept a

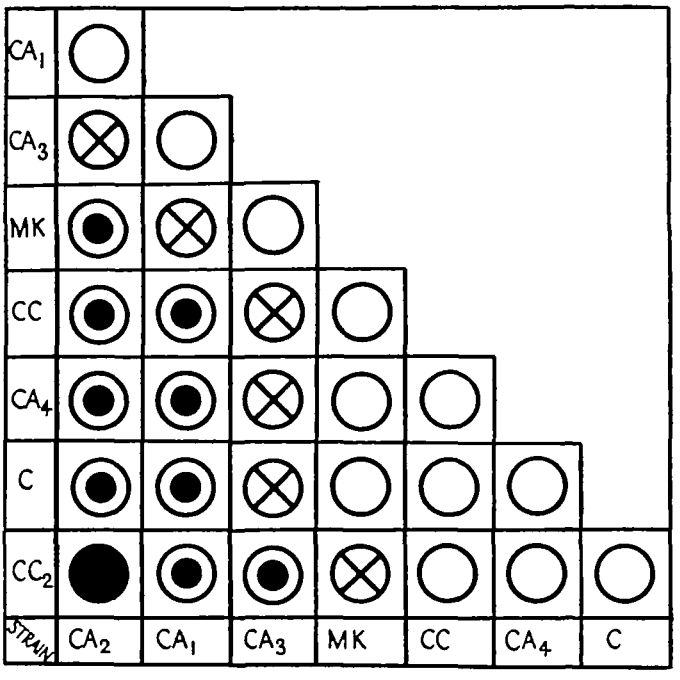

FIG. 3. Diagram showing degree of significance of difference between pairs of strains in Bramley's Seedling apple, 1928. S.E. = 0·0062. For meaning of signs see p. 202.

TABLE VIII.

Significance of the Effect of Sampling on Strains. 1928.

Mean radial advance, cm. per day.

Strain.	Sample I ($R.A._1$)	Sample II ($R.A._2$)	Difference of means.	S.E. of difference.
CA_4	0·0087	0·0274	0·0187	0·0034
CC	0·0128	0·0254	0·0126	0·0046
MK	0·0266	0·0396	0·0130	0·0054
CA_1	0·0491	0·0655	0·0164	0·0112
CC	0·0148	0·0194	0·0046	0·0046
CA_2	0·0800	0·0642	0·0158	0·0160
CC_2	0·0028	0·0019	0·0011	0·0012
CA_3	0·0523	0·0419	0·0104	0·0122

longer time in storage before estimating the amount of decayed tissue. The time-effect indicated here will be specially dealt with in a later section.

When arranged in order of attacking power, the strains show a general correspondence with the order obtained for early Bramley's in 1927. The additional strains CA_1 and CA_3 may be grouped with CA_2.

In 1929 the tests were carried out on Bramley's Seedling (from Cambridge) and Worcester Pearmain apples (from Cambridge), using the method adopted in 1927. The apples were inoculated on both sides with the same strain and the strains tested were those used in 1927. Since only a limited number of apples was available the samples used consisted of only ten individuals. After inoculation the apples were kept at room temperature (18° C.–20° C.). The rate of radial advance in cm. per day for each strain was calculated from the raw data obtained.

The full results are given in Table IX, where the strains are arranged in order of their attacking power in Bramley's. The order in Worcester is given in Roman numerals in the last column.

TABLE IX.

Bramley's Seedling and Worcester Pearmain Apples. Mean Radial Advance of Strains in cm. per day (18° C.–20° C.). 1929.

Strain.	Mean radial advance, cm. per day. Bramley's.	Worcester.	Order for Worcester.
CA_2	0·1176	0·0743	II
MK	0·0400	0·0581	III
CC	0·0391	0·0158	VI
CA_4	0·0360	0·0966	I
C	0·0273	0·0176	V
CC_2	0·0057	0·0192	IV

It is seen from Table IX that, as in previous years, the strains differ greatly in their attacking power, and that in Bramley's the grouping corresponds to that obtained for the early Bramley's of 1927 ; e.g. 'strong' CA_2; 'intermediate' MK, CC, CA_4, and C; 'weak' CC_2. In Worcester the grouping is different. The 'strong' group comprises CA_4, CA_2, and MK; the 'intermediate' CC, C, and CC_3. The 'weak' group is not represented.

The significance of the observed differences for strains, variety, and the differential effect of variety on strains (interaction) have been tested by analysis of variance, but the considerations of space do not allow of the inclusion of details of the analysis.

It was found that the value of s for strains is +1·662, which is three times the value of the 1 per cent. point (0·5522), indicating that the strains differ significantly in attacking power. The value of s for varieties is −0·5904, showing that the differences due to varieties when results for all the strains in each variety are considered together are well within the experimental error. The value of s for interactions is +1·1195, which is twice the value of the 1 per cent. point (0·6729), indicating a significant differential effect of variety on strain.

The difference between strains already brought out by analysis will now be studied in greater detail, taking into consideration the results obtained from Bramley's and Worcester separately.

Since the difference due to varieties, when all the strains are considered together, is not significant, the standard deviation (0·0267) for the entire experiment can be utilized for both Bramley's and Worcester. The standard error of difference of means will therefore be $\frac{0{\cdot}0267 \times \sqrt{2}}{\sqrt{10}} = 0{\cdot}012$. Differences of means for paired strains are presented separately for Bramley's and Worcester in Table X.

TABLE X.

Differences between Mean Radial Advance (cm. per day) of Pairs of Strains. 1929.

Bramley's Seedling Apple.

Strain.	CA_3.	MK.	CC.	CA_4.	C.
MK	0·0776				
CC	0·0785	0·0009			
CA_4	0·0816	0·0040	0·0031		
C	0·0913	0·0127	0·0118	0·0087	
CC_2	0·1119	0·0343	0·0334	0·0303	0·0216

Worcester Pearmain Apple.

Strain.	CA_4.	CA_3.	MK.	CC_2.	C.
CA_3	0·0223				
MK	0·0385	0·0162			
CC_2	0·0774	0·0551	0·0389		
C	0·0790	0·0567	0·0405	0·0016	
CC	0·0808	0·0587	0·0423	0·0034	0·0018

The degree of significance indicated by the above figures is represented diagrammatically in Figs. 4 and 5.

It is seen from Fig. 4 that in Bramley's CA_3 is significantly different from all the other strains. The remaining strains do not differ from one another.

In Worcester (Fig. 5) the following pairs of strains show no significant difference: CA_4 and CA_3; CA_3 and MK; CC_2 and C; CC_2 and CC; C and CC. The rest differ significantly from each other.

That the varietal differences in the composition of apples act differentially on the strains has been brought out by the analysis of the interaction between strain and variety. In Table XI the differences found between mean values of radial advance for individual strains in two varieties are given together with the standard error of difference of means in each case. Differences of mean values exceeding three times the S.E. of difference of means are considered significant.

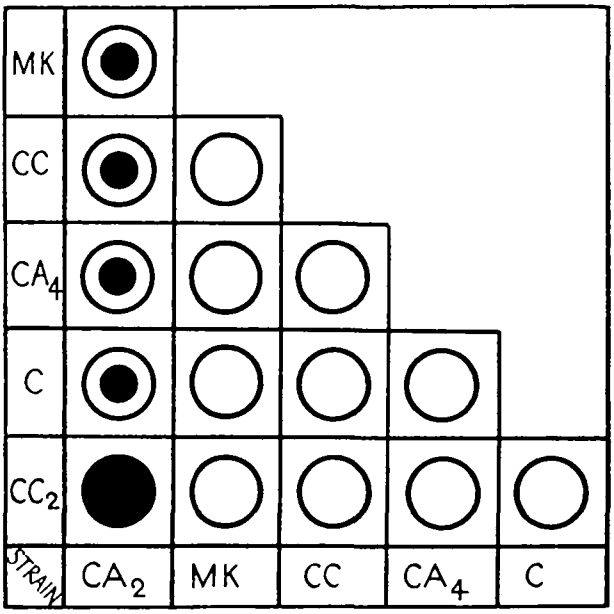

FIG. 4. Diagram showing degree of significance of difference between pairs of strains in Bramley's Seedling apple, 1929. S.E. = 0·0120. For meaning of signs see p. 202.

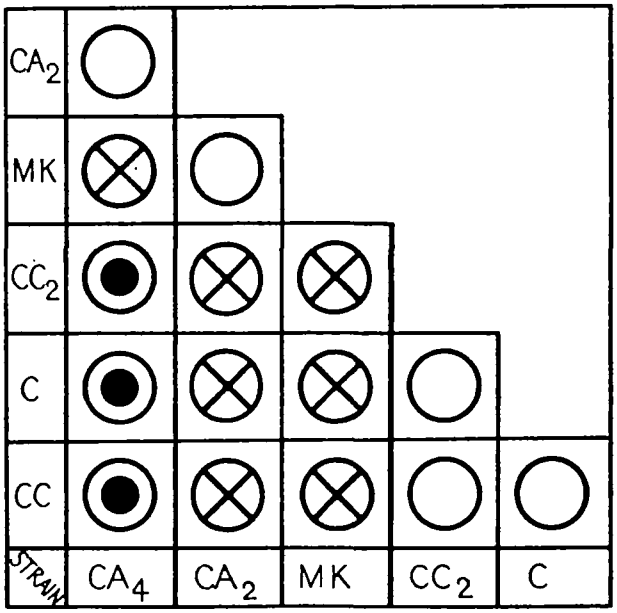

FIG. 5. Diagram showing degree of significance of difference between pairs of strains in Worcester Pearmain apple, 1929. S.E. = 0·0120. For meaning of signs see p. 202.

TABLE XI.

Significance of the Differential Effect of Varieties on Strains. 1929.

Strain.	Mean radial advance, cm. per day. Bramley's.	Worcester.	Difference of means.	S.E. of difference.
CA_4	0·0360	0·0966	0·0606	0·0101
CC_2	0·0057	0·0192	0·0135	0·0045
CC	0·0391	0·0158	0·0233	0·0087
MK	0·0400	0·0581	0·0181	0·0080
CA_2	0·1176	0·0743	0·0433	0·0226
C	0·0273	0·0176	0·0097	0·0089

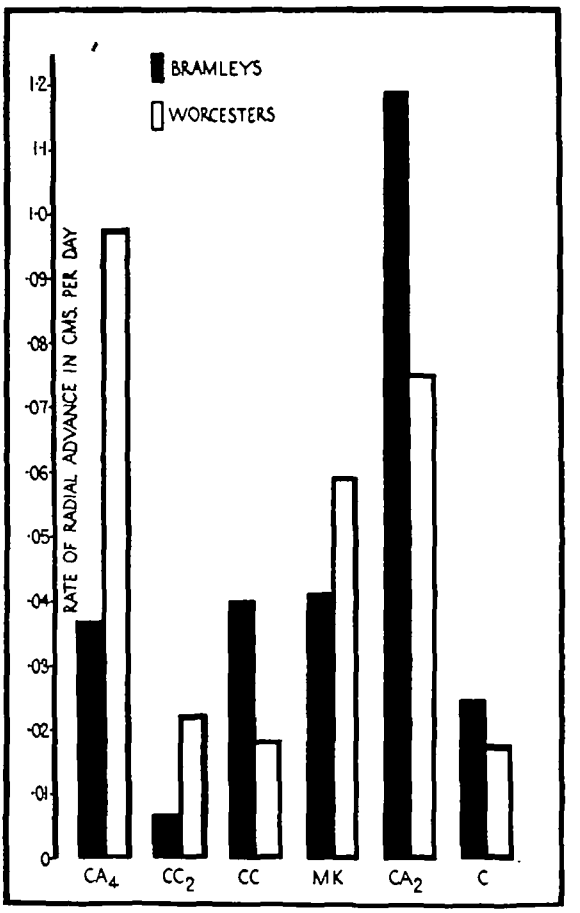

FIG. 6. Diagram showing rate of radial advance of strains of *Cytosporina ludibunda* in Bramley's Seedling and Worcester Pearmain apple, 1929.

It is seen from the table that the difference of means for CA_4 is six times the S.E. and is therefore markedly significant. With CC_2 the

difference is barely significant. The differences shown by the remaining strains are within the limits of experimental error.

These differential activities of the strains in two varieties are represented diagrammatically in Fig. 6, where the height in each column represents the radial advance in cm. per day.

It is seen that CA_4, the only strain significantly different, attacks Worcester more strongly than Bramley's, the actual rate in Worcester being three times that in the other variety. This confirms the observation of 1927, when CA_4 was found to be more active in Worcester than in Bramley's.

Among the strains (CA_2, C, CC, CC_2, and MK) which do not differ significantly in either variety, the values of radial advances recorded for MK and CC_2 are higher for Worcester than for Bramley's, but for CC, CA_2 and C the reverse relation holds.

III. Comparison of Results Obtained in 1927, 1928, and 1929.

Since the experimental conditions of temperatures and the source of apples in the first year (1927) differed from those of subsequent years, the results of all the three years could not be analysed together. It was, however, possible to analyse the results of 1928 and 1929 for Bramley's Seedling apple, since in both the years the experiments were carried out under similar conditions, apples being obtained from the same locality and inoculated with the same strains.

The samples in 1928 consisted of 18 individuals each, instead of 10 as in 1929. For analysis of variance a fresh mean was calculated for the former on the basis of ten apples in a sample. The means are given in Table XII.

Table XII.

Mean Radial Advance in cm. per day. 1928 and 1929.
(On the Basis of ten Apples per Sample.)

	Bramley's Seedling. Mean radial advance, cm. per day.	
Strain.	1928.	1929.
CA_2	0·0738	0·1176
MK	0·0340	0·0400
CC	0·0193	0·0391
CA_4	0·0184	0·0360
C	0·0124	0·0273
CC_2	0·0027	0·0057

These differences in radial advance observed in two years were tested by analysis of variance for strains, year, and for differential effect of year on strains (interaction).

The analysis showed that the value of z for *strains* is + 2·1868, while the

value of the 1 per cent. point is 0·6028. The difference among the strains is therefore to be considered very significant. The value of s for *year* is + 1·8022, about twice the value of the 1 per cent. point (0·9784), indicating that taken as a whole, the rate of attack of the strains in 1928 was significantly, different from that observed in 1929. The value of s for *interaction* (+ 1·2203) is twice the value of the 1 per cent. point (0·6028) and is therefore clearly significant. Interaction in this case will mean the complex effect of such factors as (1) Seasonal differences in the composition of apples: (2) Age of apple at the time of inoculation; (3) Possible changes in the attacking power of strains.

The effect of these combined factors on individual strains is further elucidated by the following table, where the differences between values of radial advance in Bramley's of 1928 and of 1929 are given for each strain, together with their S.E. of difference. The former should be three times the latter to be considered significantly different.

TABLE XIII.

Significance of the Differential Effect on the Strains of the Bramley's of 1928 and of 1929.

Strain.	Mean radial advance, cm. per day. 1928	1929	Difference of means.	S.E. of difference.
CA_4	0·0184	0·0360	0·0176	0·0055
CC_2	0·0027	0·0057	0·0030	0·0010
CA_2	0·0738	0·1176	0·0438	0·0205
CC	0·0193	0·0391	0·0198	0·0093
C	0·0124	0·0273	0·0149	0·0090
MK	0·0340	0·0400	0·0060	0·0085

It is seen that only CA_4 and CC_2 differ significantly in the two years.

Reference to Table XIV will show that when arranged in order of the values of radial advance in Bramley's Seedling, the strains of *C. ludibunda* fall into series which are identical in 1928 and 1929. Although the experiments in 1927 were carried out at the lower temperature of 12° C., using early and late Bramley's obtained from a different locality (Canterbury), the order of the strains approximates to that found for 1928 and 1929.

The order of the strains in Bramley's for the three years is given in Table XIV. The strains bracketed do not differ significantly from each other.

It is seen that in early Bramley's such variations in order as do occur cannot be considered significant since they involve only those strains which in the particular experiment show no significant difference from each other. In the late Bramley's of 1927 and the Bramley's of 1928, CA_4 occupies the second and fourth places respectively, while MK occupies the fourth and

the second. The difference in the order in this case must be considered significant, since the strains involved are significantly different from each other.

TABLE XIV.

Strains arranged in Descending Order of Attacking Power on Bramley's. 1927–9.

1929	1928	1927	
		Early.	Late.
CA_2	CA_2	CA_2	CA_2
MK	MK	CC	CA_4
CC	CC	MK	CC
CA_4	CA_4	C	MK
C	C	CA_4	C
CC_2	CC_2	CC_2	CC_2

The order of the strains varies with variety of apples. This is evident from the experiment carried out in 1929, in which the same strains were compared, using Bramley's Seedling and Worcester Pearmain from the same locality under similar experimental conditions. The order of attacking power in the two varieties is given in Table XV. The bracketed strains are not significantly different.

TABLE XV.

Strains arranged in Descending Order of their Attacking Power on Bramley's and Worcester. 1929.

Bramley's.	Worcester.
CA_2	CA_4
MK	CA_2
CC	MK
CA_4	CC_2
C	C
CC_2	CC

The most pronounced effect of variety is seen in the change of position of strain CA_4, which occupies the first place in Worcester and only the fourth in Bramley's. Further CC_2, a strain consistently the weakest in Bramley's, proved more active in Worcester and occupies the fourth place there. The fairly active strain CC proves the weakest in Worcester.

IV. PATHOGENICITY IN RELATION TO PEDIGREE.

It was apparent from the result of the experiment carried out in 1927 that at least one of the most active saltants was derived from a weaker parent. The experimental work of 1928 was designed to test attacking power in relation to pedigree. For this purpose the strain C, from which

all the saltants were obtained, was used together with as many descendants from the strain as were available at the time.

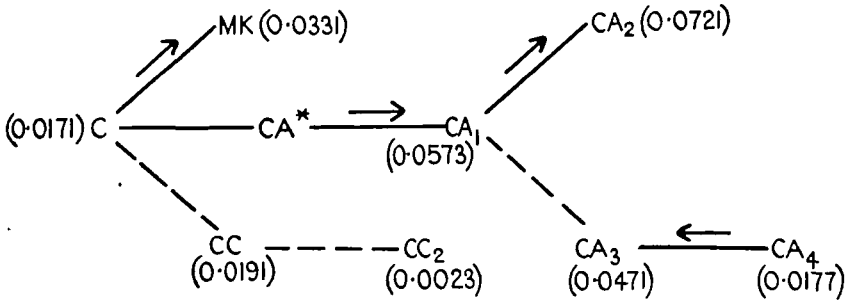

FIG. 7. Diagrammatic representation of the descent of strains of *C. ludibunda*. The mean value of radial advance (cm. per day) for each strain is given within brackets.

* Radial advance not determined.

——— Denotes significance of difference between saltant and immediate parent.

— — — Denotes no significance of difference between saltant and immediate parent.

The arrow (———→) points towards the more virulent strain.

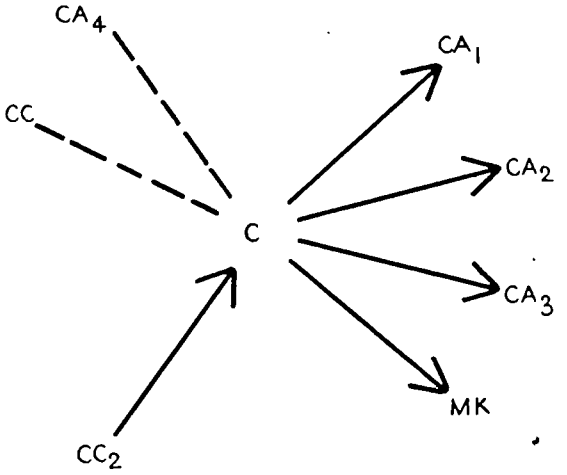

FIG. 8. *Cytosporina ludibunda*. Diagram indicating relative pathogenicity of the saltants compared with that of the parent C.

——— Denotes significance of difference between parent and saltant.

— — — Denotes no significance of difference between parent and saltant.

The arrow (———→) Points towards the more virulent strain.

The lines of descent of the strains and the mean radial advance in cm. per day in Bramley's Seedling apples for each strain is given in Fig. 7.

It is seen that the original parent, C, has given rise to a saltant MK, which is significantly more active, and to another saltant CC, from which it does not differ significantly. CC in its turn has possibly given rise to a slightly less active strain, CC_2, the difference, however, is not significant.

The saltant CA_1, derived from C via CA, is outstandingly more active than C. This CA_1 has given rise to two strains, (1) CA_2 which is more active, and (2) CA_3 which is slightly, though not significantly, less active.

CA_3 has given rise to a significantly less active strain CA_4.

When the pathogenicity of the saltants is compared with that of the original strain C (Fig. 8) it is found that the saltants CA_1, CA_2, CA_3, and MK are more virulent than C. Saltants CA_4 and CC do not differ significantly from C in virulence, and CC_2 is less virulent than C.

It should be noted that this relation between the parent strains and the saltants in regard to pathogenicity is liable to variation with alteration in the experimental factors and also with the variety of apples used. Under all conditions, however, saltants have been found which are significantly more active than the original parent C.

V. Change in Rate of Invasion with Age of Apples.

It has already been mentioned (p. 199) that the inoculation experiments of 1927 were carried out on apples gathered on two different occasions, designated early and late. Since the other experimental factors were the same the differences in the rate of invasion shown by strains in these two sets of apples should indicate the effect of age of apple on rate of invasion and, inversely, on resistance to invasion. The values of mean radial advance in cm. per day for each strain in both sets have already been given (Table II). The results are also presented graphically in Fig. 9.

It will be seen that for all the strains except CC_2 the values are greater in the late set than in the early set. The strains are, however, differentially affected by the age of the apple. For example, the radial advance of CA_4 has changed from 0·0051 cm. per day for the early set to 0·0439 cm. per day for the late, while that of C from 0·0058 cm. per day to 0·0060 cm. per day. It will also be noted that CC, which is more active than CA_4 in the early set, is less active than the same in the late set.

The significance of the observed differences between mean values of radial advance in early and late sets of Bramley's has been tested by the analysis of variance, both for the effect of age and the differential effect of age on strains (interaction).

It was found from analysis that the value of z for age is + 1·7454, about twice that of the 1 per cent. (0·9558), indicating that age of fruit has a real effect on the rate of invasion.

The value of z for interaction (+ 1·2670) is much higher than that of the 1 per cent. (0·5674), indicating a differential effect of age of apple on attacking power of strains.

This effect of age of apple on individual strains has been ascertained

separately by comparing the difference of mean values in the early and late sets for each strain with its S.E. of difference (Table XVI).

It will be observed that the difference of means for CC is about ten times and for CA_4 about four times their respective S.E. of difference. These

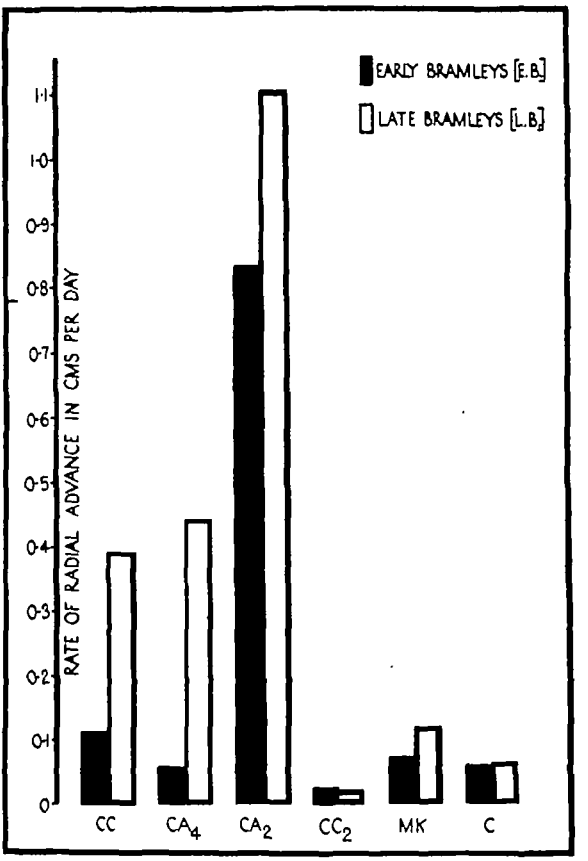

FIG. 9. Diagram showing rate of radial advance of strain *C. ludibunda* in early and late Bramley's Seedling apple at 12° C., 1927.

strains are therefore significantly different in the early and late sets. With CA_2, however, the difference falls just short of twice the S.E. of difference, therefore the chance that CA_2 is different in the late and early sets is less than 20 : 1. The difference shown by the other strains are still less significant.

It is evident from the above that the age of Bramley's apple has a definite influence on the attacking power of some strains, but may have little effect upon others. The late Bramley's are more susceptible than the early ones.

TABLE XVI.

Significance of the Differential Effect on Strains of Age of Bramley's Seedling (1927).

Strain.	Mean radial advance, cm. per day. Early.	Late.	Difference of means.	S.E. of difference.
CC	0·0109	0·0388	0·0279	0·0028
CA_4	0·0051	0·0439	0·0388	0·0090
CA_2	0·0833	0·1100	0·0267	0·0138
CC_2	0·0020	0·0016	0·0004	0·0007
MK	0·0079	0·0116	0·0037	0·0091
C	0·0058	0·0060	0·0002	0·0011

Additional data for three of the strains, CA_2, CA_4, and MK, have been placed at the author's disposal by Dr. A. S. Horne. The data in question were obtained in 1929 with Bramley's Seedling apple gathered all at one time. Sets of sixty apples, twenty for each strain, were inoculated at weekly intervals and the inoculated apples were kept at 20° C. Estimates of decayed tissue were made periodically as shown in Table XVII. In the same table is given the data of mean radial advance in cm. per day based on sixteen apples in a sample for CA_2 and CA_4 and ten apples in a sample for MK.

TABLE XVII.

Mean Radial Advance in cm. per day for CA_2, CA_4, and MK. Bramley's Seedling. 20° C. *1929.*

Samples.	Inoculated.	No. of days in store.	Radial advance, cm. per day. CA_2	CA_4	MK
A	Oct. 22	21	0·0601	0·0039	0·0126
B	,, 30	20	0·0868	0·0068	0·0172
C	Nov. 6	20	0·1340	0·0211	0·0242
D	,, 20	20	0·1853	0·0254	0·0444
E	,, 27	18	0·3090	0·0564	0·0713
F	Dec. 4	14	0·2512	0·0563	
G	,, 11	15	0·3410		

There is a gradual increase in the rate of attack with increasing age of apples, except in sample F. The significance of this observed effect of age on the rate of attack has been tested by analysis of variance, taking each strain separately.

The results in abridged form are given in Table XVIII.

1. CA_2. It will be seen from Table XVIII that the value of s for age is three times that of the 1 per cent. point, indicating that the effect of age of apple on the attacking power of CA_2 is significant.

The difference between mean values of radial advance for any two

samples are given in Table XIX, and the degree of significance shown by these differences is diagrammatically represented in Fig. 10.

TABLE XVIII.

Analysis of Variance of Change with 'Age' of Susceptibility of Bramley's to Attack by CA_2, CA_4, and MK.

Strain.	s	1 % point.	S.E. of difference of mean radial advance.
CA_2	+1·628	0·5152	0·0300
CA_4	+1·657	0·5522	0·0062
MK	+1·413	0·6472	0·0083

TABLE XIX.

Differences between Means of Radial Advance in Pairs of Samples of Bramley's Apples of Different Age. Strain CA_2.

Sample.	A.	B.	C.	D.	E.	F.
B	0·0267					
C	0·0739	0·0472				
D	0·1252	0·0985	0·0513			
E	0·2489	0·2222	0·1750	0·1237		
F	0·1911	0·1644	0·1172	0·0659	0·0578	
G.	0·2809	0·2542	0·2070	0·1557	0·0320	0·0898

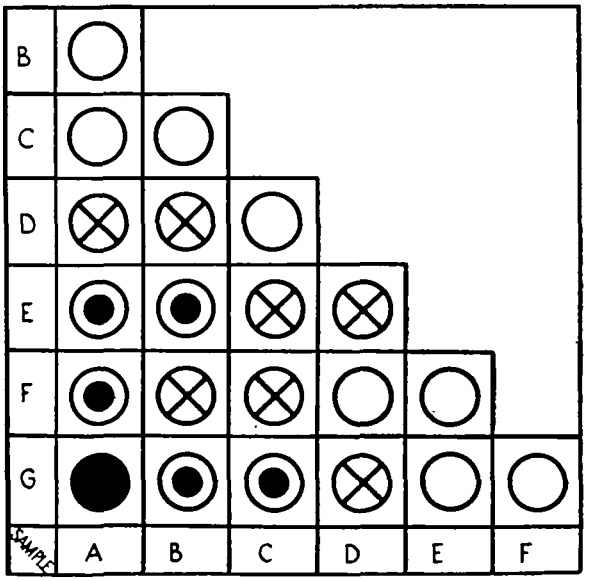

FIG. 10. Diagram showing degree of significance with age between pairs of samples of Bramley's Seedling apple inoculated with strain CA_2, 1929. S.E. = 0·0300. For meaning of signs see p. 202.

It will be observed that there is no significant difference in successive pairs of samples except in the case of samples D and E. The difference becomes significant as the difference between age of samples increases.

2. CA_4. The value of z for age is three times the value of the 1 per cent. point (Table XVIII). Hence the effect of age of apple on the attacking power of CA_4 is significant.

The significance of difference between pairs of samples is analysed below. The S.E. of difference here equals 0·0062.

TABLE XX.

Differences between Mean Radial Advance in Pairs of Samples of Bramley's Apples of Different Age. Strain CA_4.

Sample.	A	B	C	D	E
B	0·0029				
C	0·0172	0·0143			
D	0·0215	0·0186	0·0043		
E	0·0525	0·0496	0·0353	0·0310	
F	0·0524	0·0495	0·0352	0·0309	0·0001

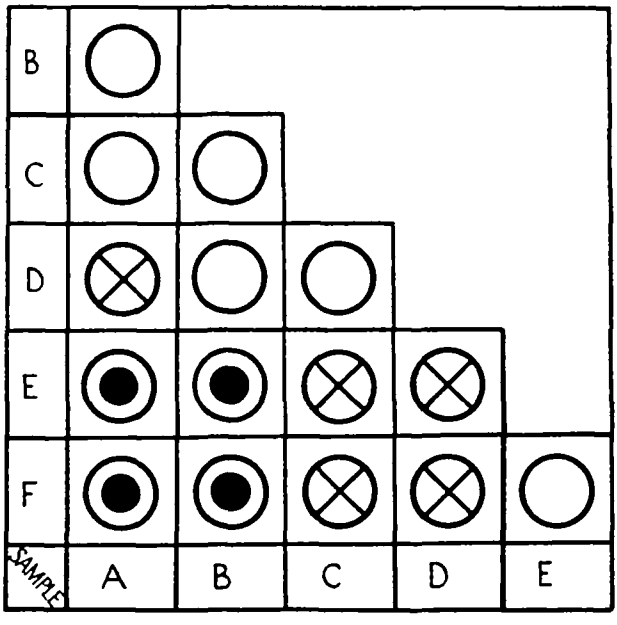

FIG. 11. Diagram showing degree of significance with age between pairs of samples of Bramley's Seedling apple, inoculated with strain CA_4, 1929. S.E. = 0·0062. For meaning of signs see p. 202.

As with CA_2, consecutive samples inoculated with CA_4 do not differ significantly, with the exception of the pair D and E.

3. MK. Table XVIII shows that the value of z for 'age is three

times that for the 1 per cent. point, and therefore the effect of age of apple on the attacking power of MK is significant.

The significance of the difference between pairs of samples is given below.

The S.E. of difference of mean radial advance is 0·0083.

TABLE XXI.

Differences between Mean Radial Advance in Pairs of Samples of Bramley's Apples of Different Age. Strain MK.

Sample.	A.	B.	C.	D.
B	0·0046			
C	0·0116	0·0070		
D	0·0318	0·0272	0·0202	
E	0·0587	0·0541	0·0471	0·0269

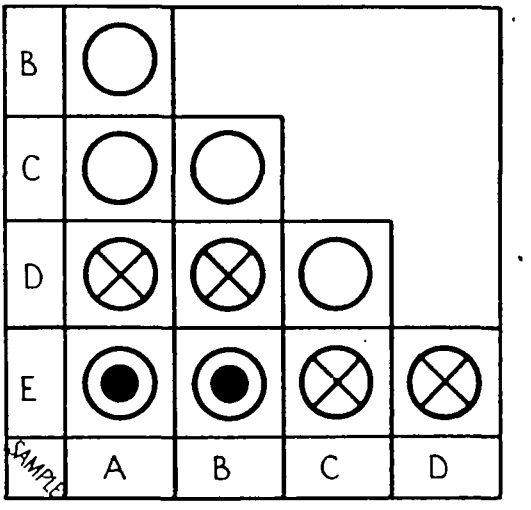

FIG. 12. Diagram showing degree of significance with age between pairs of samples of Bramley's Seedling apple, inoculated with MK, 1929. S.E. = 0·0083. For meaning of signs see p. 202.

It is seen from Fig. 12 that with MK, D and E are again the only consecutive samples which differ significantly. Significance of difference between other samples appears when differences in age are greater.

When the results of the three experiments are compared it is seen that with each strain there is significant difference in mean values of radial advance for the consecutive samples D and E, although the difference in their age is only seven days. Samples earlier and later than these, however, differ significantly only when the difference in age is three to four weeks. It is not unlikely that the age represented by samples D and E is the critical period, when the resistance of the Bramley's apple is rapidly falling.

VI. Discussion.

In 1922 Stevens (17) inoculated severed living shoots of certain cereals with seventeen saltants of *Helminthosporium*, and found them to vary in attacking power. In the following year Burkholder (3), in a paper on *Colletotrichum lindemuthianum* (Sacc et Magni) B. et C., states that the 'gamma' strain is more virulent than the 'beta' strain. The origin of the 'gamma' strain from the 'beta' strain was, however, assumed, but not established from experimental evidence. In 1925 Dickson (8) obtained a saltant of *C. atramentarium*, a fungus causing 'Black Rot' of Potatoes. It proved to be a weak parasite, but its attacking power was not compared with that of the parent strain. In 1926, Christensen and Stakman (6) recorded that the saltants which they obtained during the course of their work on *Ustilago zeae* differ greatly in pathogenicity and are less virulent than the parent. Leonian (16) in the same year stated that some saltants of *Phytophthora omnivora* are more virulent than others, but again no comparison with the parent strain was made.

Saltation resulting in the origin of strains showing increased attacking power has been recorded by Christensen (4) in 1925, who found that two mutants of *H. sativum* are decidedly more virulent than the parent with respect to both barley and wheat, especially the mutant M-40. When the attacking power of the parent was again compared with M-40 three years later the mutant proved to be less virulent than the parent.

In some of the cases mentioned above, the conclusions reached by the authors are based on very slender experimental evidence. In others the experimental results cannot be tested because the data provided are either insufficient, or of a purely qualitative nature unsuitable for statistical analysis, and hence the significance of observed differences cannot be ascertained. This is doubtless due to the difficulty in finding an accurate quantitative measure of attacking power, especially when the host material consists of either the entire plant or leaves. In the case of the apple fruit, this difficulty was overcome by Gregory and Horne, who, taking advantage of the fact that the apple is approximately spherical in shape, converted raw data of weight of rotted tissue into data of radial advance (expressed in terms of radius of apple). When the radius of the apple is known, the rate of invasion of a given fungus can be calculated. Owing to the further discovery that the values of radial advance tend to be normally distributed, it is possible to make valid comparisons of attacking power of strains by the usual method of statistical analysis.

As far as the writer is aware the statistical method was first applied to the study of the pathogenicity of strains by Horne and Gregory (15). Working with various saltants of *Fusarium fructigenum* they found that the saltants were either as virulent as, or less virulent than, the parent

strain. These results were confirmed and amplified by Harvey (11) in 1929.

Statistical methods have also been employed by Bonde (2) to compare the relative pathogenicity of the strains of *Alternaria solani.* Pathogenicity was estimated by measuring the lesion produced by the strains inoculated on potato leaves. The data were analysed by Love's modification of Student's Method, and showed that a saltant of *A. solani* (C-5) was significantly more virulent than the parent strain (C-2). The relative activity of other parent and saltant strains is difficult to ascertain from the author's account. Saltants seem to have been less virulent than the parents. The results obtained with potato tubers as host material were not treated statistically, but it appears from such information as is given that the saltant I–3, originating from a moderately virulent strain N–2, was weakly parasitic.

Statistical analysis of the data obtained in the present investigation shows that the saltants of *Cytosporina ludibunda* described in the preceding paper of this series differ greatly in attacking power. When the results obtained over three consecutive seasons are considered together, the saltants may be conveniently grouped as follows: Group I. Most active. CA_1, CA_2, and CA_3; Group II. Moderately active. MK, CA_4, CC, and C; Group III. Weak. CC_2. This grouping should not be regarded as rigid since in certain circumstances saltants placed in Group II may be found to occupy places in Group I or III.

The saltants included in Group I (CA_1, CA_2, and CA_3) are more virulent than the original strain (C) from which they were derived. In addition to these strains which proved more virulent than C irrespective of variety of apple and experimental conditions, certain other strains proved significantly more virulent than C in particular circumstances, e.g., CA_4 in late Bramley's, 1927; and CA_4 and MK in Worcester, 1929. In such cases the strains concerned would fall in Group I. It is interesting to note that the saltants in Group I (CA_1, CA_2, and CA_3) arose along one particular line of descent, viz., C–CA–CA_1, &c.

When the seasonal results obtained with a single variety of apple (Bramley's Seedling) are compared, it is seen that the order of attacking power based on estimates of the radial advance in apples which are regarded as of comparable age is not convincingly affected by season. The order in 1928 is actually the same as that in 1929. Certain differences in the order were observed, but when rigid tests were applied to the data it was found that no significance could be attached to the changes in relative position of strains. In Bramley's, CA_1, CA_2, and CA_3 were decidedly the most active strains, and CC_2 invariably proved very weak. The strains showing medium virulence differed in actual values of radial advance, but the observed differences in most cases were insignificant.

The results show that the order of attacking power is influenced by age of apple. Thus in late Bramley's, 1927, a real difference in the order was established by a significant increase in attacking power shown by CA_4, hence CA_4, which occupies a place in Group II in early Bramley's, falls into Group I in late Bramley's. A similar example of a tendency to a change in the order of attacking power with age of apple was recorded by Horne (13) for *Fusarium fructigenum*, strains D and A, in Cox's Orange Pippin. In early Cox's, *Fusarium D* was found to be much more active than *Fusarium A*, but with the increasing age of fruit the difference in activity diminished. Horne has found subsequently that the relative attacking power shown by these strains tends to be reversed with the increasing age of Worcester Pearmain apples.

The change in the observed order of strains with the increasing age of fruit is due to a differential effect of age on rate of invasion of the strains concerned. Thus with strain CA_4, mentioned above, the rate of invasion recorded for early Bramley's is 0·005 cm. per day and that in late Bramley's is 0·0439 cm. per day, whereas the corresponding rates recorded for CC are 0·0109 and 0·0388 cm. per day. With the former strain the rate has increased about eight times, and with the latter less than four times.

The general effect on rate of invasion of the increasing age of fruit (Bramley's Seedling) is shown by an analysis of data from consecutive weekly samples of inoculated apples. With every strain used the rate of invasion increases significantly with increasing age, confirming previous statements to this effect by Horne (12, 13). Thus the rate of advance of CA_2 increased nearly six times from 0·0601 (first sample) to 0·3410 cm. per day (last sample). Again, with CA_4 and MK, the rates observed for the last sample are fourteen times and seven times respectively those recorded for the first sample. The use of older Bramley's apples also seems to bring out differences between strains of *Cystosporina ludibunda* (e.g. CA_4 and CC, 1927), which cannot be detected when younger fruit is used.

Horne and Gregory (15), using Bramley's Seedling and Cox's Orange Pippin apples, found that the attacking power of strains is profoundly influenced by variety of apple. In this paper the effects of the Bramley's Seedling and Worcester Pearmain varieties on the strains of *C. ludibunda* have been compared. The results confirm those obtained by Horne and Gregory. It is found that the actual values of radial advance are different in the two varieties, especially in the case of CA_4 and CC_2, where the differences are significant. The order of the strains is also substantially altered. The most important change is shown by CA_4, which supersedes in pathogenicity CA_2, consistently the most active strain in Bramley's, and falls in the same group with it.

Analysis of the combined data obtained in 1928 and 1929 show that although all the six strains had greater rate of attack in the latter year,

only with the two CA_4 and CC_2 were the differences significant. Since the cultures of CA_4 and CC_2 showed no sign of change in the nature of their growth, it is suggested that the results of analysis indicate a differential effect of season on the attacking power of strains.

It is clear from the preceding pages that the attack of apples by the strains is affected by age and variety of apple and perhaps to a certain extent by season. These factors are known to affect the chemical constitution of the apple (1) and the evidence suggests that the observed variations in rate of invasion are due to such changes in composition of fruit rather than to modification of the fungal strains.

VII. Summary.

The attacking power of certain saltants of *Cytosporina ludibunda* described in the second paper of this series, has been tested and compared with that shown by the parent strain by inoculating apples and making estimates of the rate of invasion of the tissues. The experimental work has extended over three years (1927–9). The significance of the observed differences has been tested by the usual methods of statistical analysis.

Certain saltants (CA_1, CA_2, CA_3) proved to be more active than the parent, C, under the experimental conditions employed; others (CC, CA_4, MK) usually showed a degree of activity similar to that of the parent, but under certain experimental conditions (apple variety) CA_4 and MK were more active than C. Strain CC_2 proved to be, on the whole, the weakest strain tested. The rate of invasion varied greatly, the most active strain being from 20 to 50 times more active than CC_2.

Both variety of apple and age of fruit have a differential effect on attacking power of strains. A differential effect of season on attacking power of strains is suggested by the evidence, but is not regarded as conclusively established.

It is clear that differences or changes in resistance of apples to invasion (probably conditions of chemical composition) rather than modification of the fungus, are responsible for the observed variation in rate of radial advance with variety and age of apple.

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LITERATURE CITED.

1. ARCHBOLD, H. K.: Chemical Studies in the Physiology of Apples. IX. The Chemical Composition of Mature and Developing Apples, and its Relationship to Environment and to the Rate of Chemical Change in Store. Ann. Bot., xlii, 550–7, 1928.
2. BONDE, R.: Physiological strains of *Alternaria Solani*. Phytopath., xix, 533–48, 1929.
3. BURKHOLDER, W. H.: The Gamma Strain of *Colletotrichum lindemuthianum* (Sacc. et Magn.) B. et C. Phytopath., xiii. 316–23, 1923.
4. CHRISTENSEN, J. J.: Physiologic Specialization and Mutation in *Helminthosporium sativum*. Phytopath., xv. 785, 1925.
5. ————————: The Influence of Temperature on the Frequency of Mutation in *H. sativum*. Phytopath., xix. 155–62, 1929.
6. ————————, and STAKMAN, E. C.: Physiologic Specialization and Mutation in *Ustilago zeae*. Phytopath., xvi. 979–99, 1926.
7. DAS GUPTA, S. N.: Studies in the Genera *Cytosporina*, *Phomopsis*, and *Diaporthe*. II. On the Occurrence of Saltation in *Cytosporina* and *Diaporthe*. Ann. Bot., xliv. 349–84, 1930.
8. DICKSON, B. T.: Further Studies on Saltation in the Organism causing 'Black Dot' disease of Potato. Trans. Roy. Soc. Canada, 3rd series, xix. 275–7, 1925.
9. FISHER, R. A.: Statistical Methods for Research Workers: London, 1930.
10. GREGORY, F. G., and HORNE, A. S.: A Quantitative Study of the Course of Fungal Invasion of the Apple Fruit and its Bearing on the Nature of Disease Resistance. Part I. A Statistical Method of Studying Fungal Invasion. Proc. Roy. Soc., B, cii. 427–43, 1928.
11. HARVEY, C. C.: Studies in the Genus *Fusarium*. VII. On the different degrees of parasitic activity shown by various strains of *F. fructigenum*. Ann. Bot., xliii. 245–59, 1929.
12. HORNE, A. S.: Changes in the Resistance of the Apple Fruit to Fungal Invasion. Report of the Food Investigation Board. 96–107, 1928.
13. ————————: Changes in the Resistance of the Apple Fruit to Fungal Invasion. Ibid 125–44, 1929.
14. ————————, and DAS GUPTA, S. N.: Studies in the Genera *Cytosporina*, *Phomopsis*, and *Diaporthe*. I. On the Occurrence of an 'Ever-saltating' strain in *Diaporthe*. Ann. Bot., xliii. 417–35, 1929.
15. ————————, and GREGORY, F. G.: A Quantitative Study of the Course of Fungal Invasion of the Apple Fruit and its Bearing on the Nature of Disease Resistance. Part II. The Application of the Statistical Method to Certain Specific Problems. Proc. Roy. Soc. B, cii. 444–66, 1928.
16. LEONIAN, L. H.: The Morphology and Pathogenicity of some *Phytophthora* Mutations. Phytopath., xvi. 723–30, 1926.
17. STEVENS, F. L.: The *Helminthosporium* Foot Rot of Wheat, with Observations on the Morphology of *Helminthosporium* and on the Occurrence of Saltation in the Genus. Dept. Regist. and Educ. Div. Nat. Hist. Survey, Illinois, xiv. 126–36, 1922.