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.*

By F. G. Gregory, D.Sc., and A. S. Horne, D.Sc.

(Communicated by Prof. V. H. Blackman, F.R.S.—Received March 31, 1927.)

(From the Department of Scientific and Industrial Research, and the Department of Plant Physiology and Pathology, Imperial College of Science and Technology, London.)

CONTENTS.

I.—*Introduction.*

In the course of investigations on the incidence of disease in apples stored at low temperatures, which have been in progress since 1920, it soon became evident that a regular series of fungal species appeared successively during the period of storage. It seemed desirable to investigate the problem more closely with a view to disentangling the factors concerned in the succession. The first method employed for the purpose consisted in taking a periodical census of the population of apples and noting the number of apples "invaded" and "not invaded." The need for improvement on this method of studying fungal invasion became clear in the course of the investigation, and led to the development of the technique, described in the present paper. The method of the periodical census has been utilised by M. N. Kidd (1) who has constructed curves of the progress of fungal invasion and from them has derived "mortality curves." The interpretation of these "mortality curves" is, however, somewhat difficult, representing, as they do, not only the effect of varying resistance in the apple, but also the interaction of this factor with the chances of casual infection, which includes the varying powers of penetration possessed by different species of fungi. The distribution of the two classes of apples "invaded" and "not invaded," depends on the interaction of three factors:

(1) the conditions of infection, (2) the progress of invasion after infection, and (3) the final stage of invasion. The bearing of these factors on the problem of invasion of apples will now be briefly considered.

(1) Unless precautions are taken to ensure equal chances of infection by utilising a sound technique of inoculation the problem of the incidence of disease in stored apples is confused by factors such as the unequal distribution of infecting organisms in individual apples of the population, the unequal distribution of spores on individual apples in relation to the distribution of the lenticels or other points of entry, and so on. The method employed in the course of this investigation has already been described (2) and has proved itself adequate.

(2) It is the problem of the progress of disease after infection which will be mainly dealt with, and the method of study will be fully described subsequently in this paper. It is clear, however, that the method previously employed takes no account of the degree of infection, and indeed the assessment of the virulence of two species or strains of fungi will depend on the time at which the census is taken, and should the onset of the invasion be much delayed after the entry of the fungus the main progress of the invasion may be altogether missed.

(3) In the course of the investigation it became clear that after the primary infection the further development of the fungus may be arrested for a long period of time although the fungus remains viable. Such arrest of invasion, which has been called "interference" in this paper, was previously noticed in studying the "spotting" of apples (3) and has been met with on several occasions in the course of this work. This "interference" must be taken into account in assessing the virulence of particular species or strains. The phenomenon is always confined to a varying proportion of the total number of apples in which rotting is checked in the early stages of invasion, while in the remainder invasion proceeds normally to completion. Unless the course of the invasion is followed in such cases an entirely erroneous conclusion will be reached, since by mere inspection apples showing "interference" would be placed in the class "not invaded."

II.—*The Material Used.*

The varieties of apples used throughout this investigation were Cox's Orange Pippin from Burwell, Cambridgeshire and Bramley's Seedling from six localities.

The fungi principally used were originally isolated from apples as previously described (3, 4) and consisted of *Botrytis* sp., *Cytosporina ludibunda*, *Fusarium* sp. (5), *Pleospora pomorum* and *Polyopeus aureus*.

III.—*The Experimental Method.*

At the time of gathering, apples were selected as far as possible of uniform size and free from obvious blemishes. They were then infected at two opposite equatorial points by the standard method already described (2) and transferred as soon as possible to the Low Temperature Station, Cambridge, where they were kept under constant conditions of temperature and humidity. For this work two temperatures were mainly employed, viz., 3° C. and 12° C. From time to time batches of inoculated apples were despatched from Cambridge to London for examination. The apples were then individually weighed and the superficial diseased areas on the two sides were either measured, or the diseased tissue removed and weighed. The measurements taken of the two diseased areas were the mean diameters, obtained by averaging the lengths of two diametral arcs at right angles. The weight of the diseased tissue was obtained by carefully scooping out the decayed areas on each side in turn and weighing the apple after each operation. When invasion was nearly complete it was not possible to record the weights in the two sides separately.

In cases where "interference" had occurred it was found that the rotten tissue had dried out and hollow spaces were left inside the apple. In order to estimate the extent of the invasion prior to drying in such cases, the volume of the hollow was measured by filling with water from a burette and so obtaining the volume. The specific gravity of the apple tissue was then determined and these volumes were converted into weights.

In all doubtful cases where contamination was suspected the causal organism was re-isolated, and unless the original infecting fungus was recovered unassociated with others, the apple was rejected.

The results here recorded are based on a selection from experimental data involving 15,000 separate inoculations, which were carried through by competent helpers.* The main body of results will be dealt with in a second paper (Part II).

IV.—*A Method of Treating the Primary Data for Statistical Purposes.*

The kinds of problems which will be dealt with in the second part of this paper are as follows :—

(1) Comparison of the infecting power of different species and strains of fungi in apples of a given variety.

* The authors' thanks are due to Mrs. Cartwright, Mrs. Harvey and Mrs. Horne for the skilful and efficient way in which this work was done.

(2) The resistance to invasion of different varieties of apples to certain fungal species and strains.

(3) The change in resistance undergone by apples during storage and during the course of invasion by fungi.

The infecting power of the fungi must be assessed from the amount of damage done in a given time, or the time taken to do equivalent amounts of damage. Three possible measures of the damage done will be considered: (*a*) the superficial area of discoloration; (*b*) the weight of rotten tissue produced in a given time, either as absolute weight or as percentage of the weight of the apple; (*c*) a measure derived from the weight of rotten tissue, which will be called "radial advance."

The method of assessing the amount of damage by the measurement of superficial areas was used for one experiment only, before the weighing method had been tried. It was abandoned, since it was found that the area of discoloration apparent on the surface was in many cases smaller than the real affected area, a thin superficial layer of sound tissue being left just below the skin.

The weight of rotten tissue may be accurately determined, but the following considerations indicate, however, that the weight of rotten tissue is not an ideal measure to employ.

It has been assumed that infection proceeded from two points at the opposite ends of a diameter in the equatorial plane of the apple, and that the advance has followed spherical shells centred at the points of infection. The further assumption has been made that the apple is spherical in form and is uniform throughout. If the apple were a sphere of unlimited size and invasion proceeded at a uniform rate then the mass of decayed tissue would increase as the cube of time.

Actually the apple approximates to a sphere of very limited size compared with the infected volume and hence only in the very early stages would this relation hold. The curve of total mass of decayed tissue plotted against time is in fact of complex sigmoid form. It can be shown by geometry that in a sphere of radius R, when the front of the invaded zone has reached a certain distance from the point of inoculation, where x is the distance divided by the length of the radius of the sphere, the volume (V) of affected tissue may be expressed by the equation

$$V = \frac{\pi}{12} R^3 x^3 [8 - 3x],$$

when $x = 2$ this becomes $V = \frac{4}{3}\pi R^3$ and is the total volume of the sphere. The distance from the point of inoculation to the front of the invasion, expressed as

a fraction of the radius, will subsequently be called "radial advance." Taking the total volume of the sphere as 1, the proportion (v) of the total volume affected will be given by

$$v = \frac{x^3(8 - 3x)}{16}.$$

This relation holds only when invasion proceeds from one point on the surface of the apple. When two points of infection are considered, the proportion of the total volume affected will be double the amount calculated from the formula in the early stages, until the advancing fronts of invasion meet at the centre of the sphere, when 63 per cent. of the total volume has been invaded. After

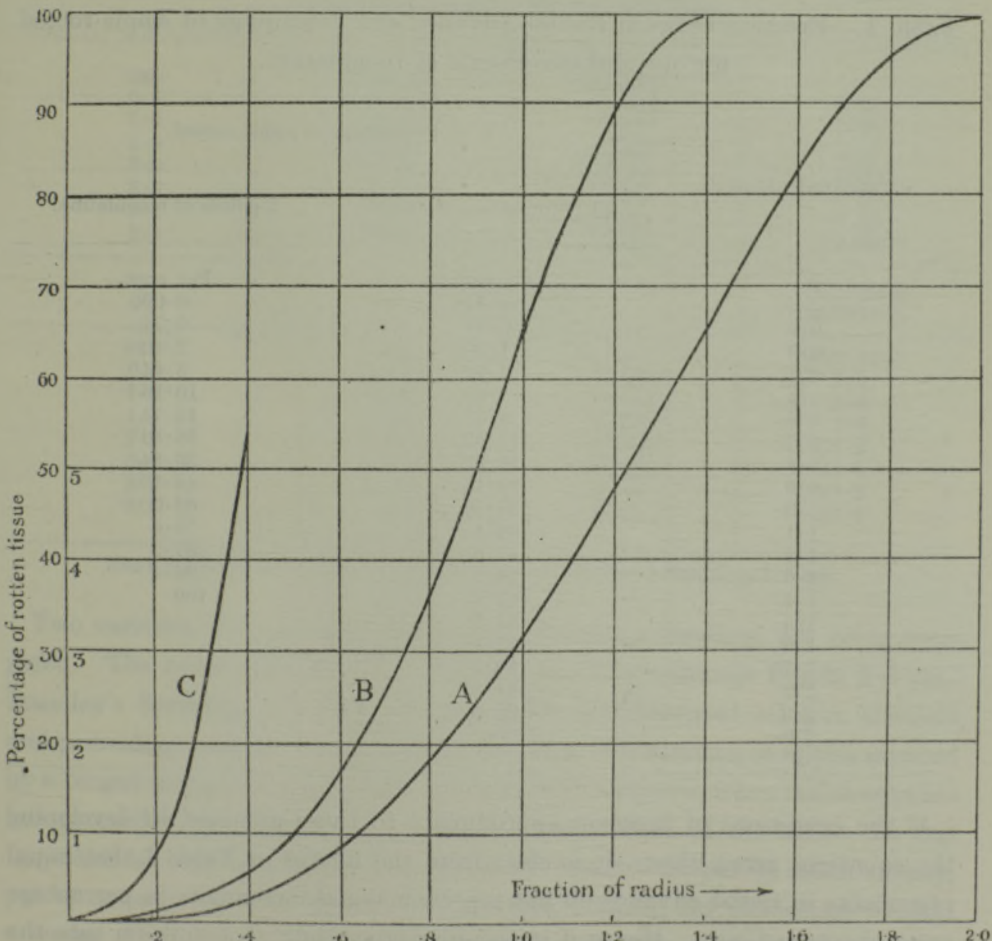

FIG. 1.—Curve showing the relation between the percentage of rotted tissue and radial advance, expressed as a fraction of the radius. A, for apples inoculated at one point. B, for apples inoculated at two points. C, early part of curve B enlarged 10 times.

this, overlapping will occur and a correction has to be made for the volume of overlap, as proportion of total volume of sphere, given by the expression

$$v' = \frac{(x - 1)(4x^2 - 2x - 2)}{4}.$$

Fig. 1 shows the curve of percentage volume of mass rotted (v) plotted against x (x = radial advance) for a sphere inoculated at one and two points respectively, and in Table I the values are given of the percentage mass (V) rotted for increasing radial advances. It is evident that complete rot corresponds to a penetration of 1·4 ($= \sqrt{2}$) times the radius.

Table I.—Relation between Radial Advance and Percentage of Apple rotted for one and two Points of Inoculation.

x = Radial advance.	V = Percentage of apple rotted.	
	1 point of inoculation.	2 points of inoculation.
	Per cent.	Per cent.
0·1	0·048	0·096
0·2	0·37	0·74
0·3	1·20	2·40
0·4	2·72	5·44
0·5	5·08	10·16
0·6	8·37	16·74
0·7	12·65	25·30
0·8	17·92	35·84
0·9	24·15	48·30
1·0	31·50	63·00
1·1	39·1	76·6
1·2	47·5	87·2
1·3	56·3	96·4
1·4	65·2	100
1·5	73·8	
1·6	82·4	
1·7	89·1	
1·8	94·8	
1·9	98·6	
2·0	100	

If the conditions of invasion approximate to those assumed in developing the equations given above, it is clear from the figures in Table I that equal increments in radial advance do not represent equal increments in percentage mass of rotten tissue. Hence if invasion is proceeding at a uniform rate the relation of percentage amount of rot, hence also the weight of rotten tissue, produced in two equal increments of time will depend on the particular stage of invasion at which the comparisons are made. Further the relations of amounts

of rotten tissue will depend on the size of the apples inoculated, and if relative virulence is estimated by comparison of weights of tissue rotted, errors will be introduced unless a correction for size of apples is made. An example of the magnitude of the errors liable to be introduced by the effect of size of apple is shown in Table II.

Table II.—Errors introduced into Comparison of Invasion by Effect of Size of Apple.

Radial advance in cm.	Percentage weight of rot. Cox's Orange Pippin.	Percentage weight of rot. Bramley's Seedling.	Ratio.
cm.	Per cent.	Per cent.	
0·5	0·656	0·254	2·58
1·0	4·88	1·92	2·54
1·5	15·05	6·10	2·47
2·0	32·38	13·58	2·38
2·5	56·84	24·78	2·29
3·0	87·14	39·80	2·19
3·5	98·62	58·40	1·69
	Weight of rot.		Percentage difference.
cm.	cm.	cm.	Per cent.
0·5	0·115	0·119	3·4
1·0	0·858	0·896	4·4
1·5	2·645	2·846	7·6
2·0	5·691	6·336	11·3
2·5	9·990	11·561	15·7
3·0	15·32	18·57	21·2
3·5	17·33	27·25	57·2

Mean radius Cox's Orange Pippin, 2·6 cm.; Bramley's Seedling, 3·6 cm.

Two varieties, Cox's Orange Pippin and Bramley's Seedling, are being compared. The mean radii for these varieties are Cox's Orange Pippin 2·6 cm., Bramley's Seedlings 3·6 cm. In the table are presented relative absolute and percentage weights of rotten tissue for these two varieties of apples invaded by a fungus assumed to be equally active in both varieties, when radial advance has proceeded for distances given in the first column of the table. In this hypothetical case the fungus is known to be equally virulent in either variety, yet by comparing either the weights of rotten tissue or the percentage weights a wrong estimate of virulence would result, and the errors involved would depend on the stage of invasion at which comparison was made.

This effect of absolute size of apples invalidates the use of absolute or percentage weights of rotten tissue in comparing the virulence of a given species

of fungus in apples of the same or different varieties if these differ in absolute size. An ideal measure of invasion should be independent of the size of the apple, and moreover should give equal numerical values for equal intervals of time, if the rate of invasion remains constant. In so far as the assumptions made in deriving the radial advance from the percentage weight of apple affected are justified, radial advance will successfully eliminate the effect of absolute size, and will give equal numerical values for equal intervals of time if the rate of invasion of the fungus is constant. The primary data derived from weight of rotten tissue can therefore by conversion to radial advance be used to compare the course of invasion by fungi even where the rate of invasion differs in two cases, or apples of different sizes are used. Further, any change in resistance will be reflected in a rise or fall in magnitude of the rate of radial advance, and in no other way could changes in resistance during invasion be followed.

The method of dealing with samples which was finally adopted was as follows. The sum of the weights of rotted tissue recorded from the two sides was first converted into percentage of the total weight of the apple. From the curve in fig. 1 the percentage data were converted into percentages of radial advance. From the weights of the individual apples and the specific gravity characteristic for the variety of apple, the mean radius in centimetres of the apples constituting the sample was calculated, and this figure multiplied by the mean radial advance measured the penetration in centimetres. In this way apples of different sizes could be compared and the rate of invasion calculated over different periods of time and with different fungal species. The probable error of the figures obtained from each operation was calculated, and hence the probable errors of the final figures for the rate of invasion were known.

V.—*The Distribution of Radial Advance and Percentage Weight of Rotted Tissue in Random Samples of Apples.*

In practice the progress of invasion in a uniform set of apples by a given species of fungus is followed by taking samples at different times and determining the amount of rotten tissue in each apple by the method of weighing already indicated. Although the apples are originally selected as uniform as possible, yet in any one sample large variations in weight of rotten tissue from the individual apples are invariably found. These differences in the amount affected may be attributed to variations in resistance to invasion in individual apples. Considering two apples of different resistance inoculated with the same fungus, it may be assumed that the radial advance will be inversely proportional to the

resistances of the two apples.* From the distribution of radial advance in a set of apples some information may be derived as to variation in resistance in individual apples in a sample. Since the resistance cannot be directly measured, its nature being unknown, and its magnitude being assumed to be inversely proportional to rate of radial advance, any conclusions as to resistance will depend for their value on the validity of the assumptions made in deriving radial advance from the experimental data of weights of rotten tissue. It may be well therefore to present evidence that the distributions of weights of rotten tissue in samples of apples of varying resistance actually conform with those expected if the conditions of invasion approximate to those postulated in the geometrical treatment of the invasion of a uniform sphere.

In fig. 2 is presented a set of distribution curves embodying the data from five

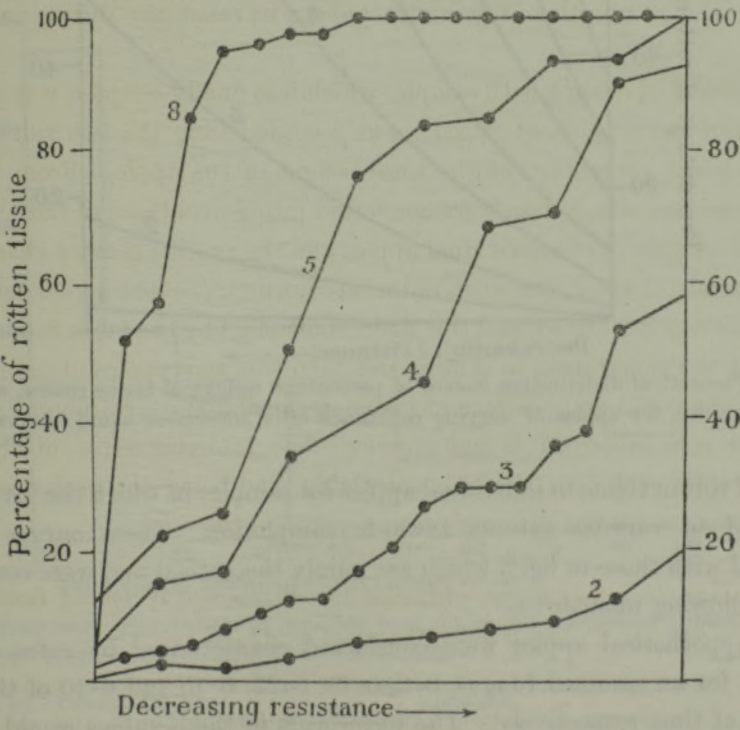

Fig. 2.—Distribution curves of percentages of weight of tissue rotted in samples of apples arranged in serial order of increasing weight, for comparison with the theoretical distributions in fig. 3.

2 = Cox's Orange Pippin 12° C., 1924–25. *Fusarium* strain B11.
3 = Bramley's Seedling, 12° C., 1924–25. *Fusarium* strain C21.
4 = Cox's Orange Pippin, 12° C., 1924–25. *Fusarium* strain A.
5 = Cox's Orange Pippin, 12° C., 1924–25. *Fusarium* strain C1.
8 = Cox's Orange Pippin, 12° C., 1925–26. *Botrytis* sp.

* This will only be true up to the limit at which the fungus is growing at the maximum rate conditioned by internal factors.

selected experiments. The ordinates are the percentage weights of rot from each individual apple in the sample, which have been arranged in serial order along the abscissa. The various curves indicate the distribution of percentage

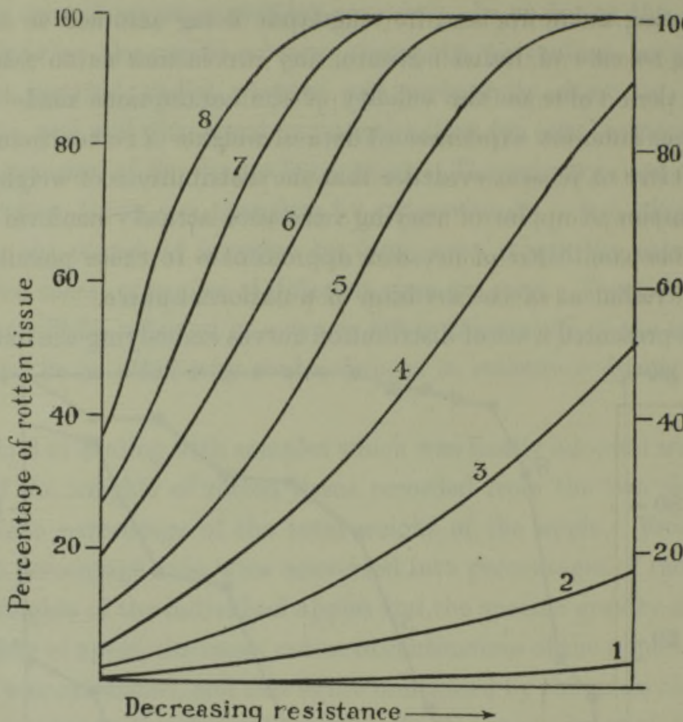

FIG. 3.—Theoretical distribution curves of percentage weight of tissue rotted, arranged in serial order, for apples of varying resistance after successive equal intervals of time (1–8).

weight of rotten tissue in individual apples for samples in which the invasion has proceeded to varying extents towards completion. These curves may be compared with those in fig. 3 which are purely theoretical and were constructed in the following manner :—

Five hypothetical apples were considered characterised by rates of radial advance, for an assumed fungus, 0·3, 0·25, 0·20, 0·15 and 0·10 of the radius per unit of time respectively. The reciprocals of these values would give the relative magnitudes of the resistances of these hypothetical apples. The apples have been arranged in serial order of increasing radial advance, the apple of highest resistance being on the left of the diagram, the others being placed in order of decreasing resistance. Taking times 1, 2, 3, 4, etc., the radial advances after successive periods of time were found, and from the curve in fig. 1 these values of radial advance were converted into the corresponding percentages of rotten tissue.

It is the values of percentage of apple rotted which are presented as curves in fig. 3, each curve being marked with the period of time elapsed. The curves thus represent distribution curves of weight of rotted tissue, arranged in serial order for a given set of apples, after the lapse of varying periods of time; or they may represent equally well distribution curves which would be obtained after a given period of time from sets of apples, all of the same kind, but infected with fungi of different virulence. The resemblance of the theoretical and experimental distribution curves* lends some probability to the validity of the simplifying assumptions introduced into the geometrical considerations of the problem of invasion. Frequency distributions of percentage weight of rotten tissue and of radial advance in a large population of apples are shown in figs. 4 and 5.

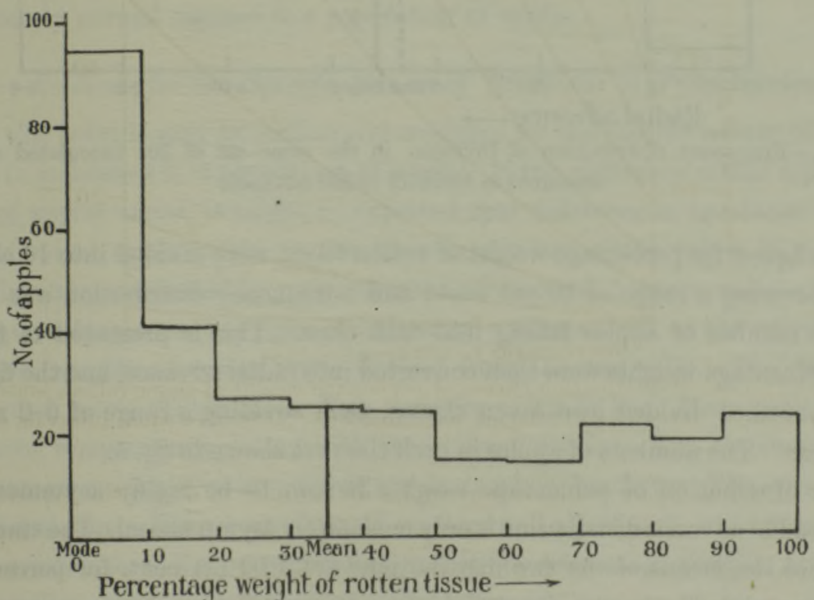

Fig. 4.—Frequency distribution of invasion in a set of 301 inoculated apples, measured in terms of percentage weight of rotted tissue.

Cox's Orange Pippin { 3° C., 1925–26 ; 12° C., 1925–26 } *Botrytis.*

" " { 3° C., 1925–26 ; 12° C., 1926–27 } *Cytosporina.*

Bramley's Seedling { 12° C., 1926–27 ; 20° C., 1926–27 ; 20° C., 1927–28 } *Cytosporina.*

* A rigid test of the similarity of the curves in figs. 2 and 3 would require an independent measure of the resistance in individual apples. The only assumption which has been made is that resistance does vary in individual apples, and that the individuals in a sample can be arranged in a serial order of increasing resistance.

The data were derived from 301 apples in five samples, three sets of Cox's Orange Pippins (177 apples), and two sets of Bramley's Seedlings (124 apples).

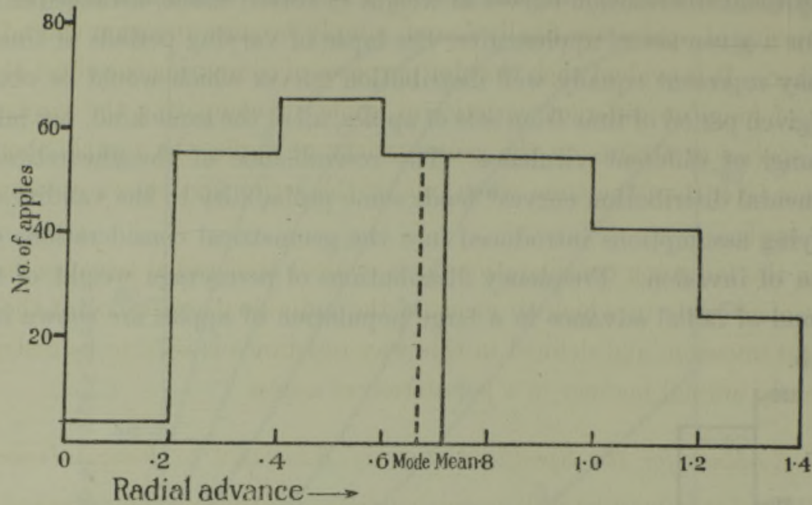

Fig. 5.—Frequency distribution of invasion in the same set of 301 inoculated apples measured in terms of radial advance.

The figures for percentage weight of rotten tissue were divided into 10 classes each covering a range of 10 per cent., and a frequency distribution was made of the number of apples falling into each class. This is presented in fig. 4. The percentage weights were then converted into radial advance, and the figures thus obtained divided into seven classes, each covering a range of 0·2 radial advance. The numbers of apples in each class are shown in fig. 5.

The distribution of percentage weights is seen to be highly asymmetrical, while radial advance distribution is only moderately asymmetrical. The standard errors of the means of the two distributions are 5·02 per cent. for percentage weights and 2·23 per cent for radial advance respectively; hence mean radial advance of a sample may be estimated with twice the accuracy of percentage weight of rotten tissue. In addition, therefore, to the advantage which radial advance has over weight of rotten tissue as a measure of invasion in eliminating the effect of size of apple and the stage of invasion reached, the added advantage of greater accuracy is obtained, so that samples of one-half the size may be used without loss of efficiency. Since distributions departing largely from symmetry are statistically inconvenient, the treatment of the results of the experiments to be reported in Part II of this paper would seem to require the choice of radial advance as the working variate.

The distribution of radial advance itself, however, tends to become increasingly

asymmetrical as invasion proceeds towards completion. Increasing numbers of apples will fall into the class 100 per cent. rotten (radial advance 1·4), and the mode of the distribution will shift gradually towards the highest class, in which the majority of apples are finally found, simply because invasion can advance no further. It is evident then that for purposes of comparing the effect of given species of fungi on different varieties of apple, or comparing the virulence of fungal species or strains on the same variety of apple, the sample should be taken not later than the time when one of the series first shows a completely rotten apple.

From the foregoing considerations it is evident that radial advance provides a convenient scale in which to measure the individual resistance of the apple to fungal invasion, and defined in this way resistance is seen to be distributed in a nearly normal manner in a population of apples.

VI.—*Evidence for the Specific Nature of Resistance to Fungal Invasion.*

At this point it may be well to give evidence for the specific nature of variations in resistance in a population of apples. If the resistance within a population of apples varies, it might be expected that difference in resistance within an individual apple would be less than in any two apples taken at random. This has been confirmed by estimating the correlations between radial advance of a given fungus on two sides of individual apples as well as the correlations between weights of rotten tissue derived from the two sides. The data are presented below (Table III). Two varieties of apple have been used at two different temperatures, tested with three fungal species. The correlations for percentage weight of rotten tissue are enclosed in brackets. The correlation coefficients for radial advance are all positive and significant, and are in every case greater than the corresponding values for percentage weight, reflecting the smaller standard deviation of radial advance.

The correlations provide clear evidence of the specific value of resistance in individual apples.

Although the correlations so far presented indicate that the resistance at one pole of an apple tends to be similar to that at the opposite pole, examination of a set of data giving individual weights of the rotten tissue from the two sides reveals striking differences. It seemed possible therefore that the resistance of an individual apple might vary greatly from point to point around the circumference.

To test this point a set of 20 Bramley's Seedling apples was taken and each apple was inoculated at four points equidistant around the circumference. The

results as determined in terms of radial advance after storage for 17 days are given in Table IV.

Table III.—Correlation between Estimates of Invasion on two Sides of Individual Apples.

Season.	Fungus.	Temperature.	Variety of apple.	Correlation coefficient (r).	No. of apples.
		° C.			
1925–26	*Fusarium*. Strain D	12	Bramley's Seedling	(+0·603) +0·874	19
1925–26	*Botrytis*. Sample I	3	Cox's Orange Pippin	(+0·479) +0.598	20
1925–26	*Botrytis*. Sample II	3	Cox's Orange Pippin	(+0·597) +0·655	16
1925–26	*Cytosporina*. Sample II	3	Cox's Orange Pippin	(+0·428) +0·657	16
1925–26	*Cytosporina*. Sample I	3	Cox's Orange Pippin	(+0·610) +0·662	20
1924–25	*Cytosporina*	3	Bramley's Seedling	(+0·556) +0·717	25
			Mean	(+0·546) +0·694	116

Table IV.—Radial Advance at four equidistant Points in Individual Apples Inoculated with same Fungus.

	a.	*b*.	*c*.	*d*.
1	0·26	0·25	0·22	0·28
2	0·28	0·24	0·24	0·27
3	0·41	0·30	0·37	0·38
4	0·37	0·27	0·28	0·23
5	0·41	0·28	0·26	0·27
6	0·43	0·28	0·28	0·29
7	0·39	0·26	0·27	0·30
8	0·37	0·22	0·36	0·24
9	0·44	0·18	0·38	0·33
10	0·47	0·28	0·38	0·28
11	0·54	0·40	0·48	0·29
12	0·38	0·30	0·32	0·28
13	0·46	0·33	0·36	0·32
14	0·50	0·28	0·36	0·28
15	0·46	0·28	0·40	0·36
16	0·60	0·56	0·45	0·40
17	0·54	0·42	0·44	0·37
18	0·41	0·37	0·28	0·33
19	0·52	0·39	0·28	0·39
20	0·62	0·28	0·38	0·42
Mean	0·443	0·309	0·340	0·316

In each case the figures are the radial advances occurring at the four points (*a*, *b*, *c*, *d*), the highest value recorded in each case being placed first (*a*), and the rest in serial order around the circumference. The mean values show an alternation of high and low values. The mean value of the figures in column *c* is necessarily lower than the mean value of those in column *a*, since the highest values have arbitrarily been selected and placed first. From these figures it is possible to test more rigidly the relation of the resistances of opposite sides of individual apples.

In order to test this point correlations were calculated between radial advance in column *a* and the values of each of the other columns in turn. The results are as follows :—

$$\left.\begin{array}{l} r_{ab} = +0\cdot680 \\ r_{ac} = +0\cdot742 \\ r_{ad} = +0\cdot679 \end{array}\right\}.$$

All these correlations are positive and highly significant, indicating again that the resistance within an apple tends to a specific value. To test whether the alternation in high and low values in columns *b*, *c* and *d*, as indicated by the means, is significant, the effect of the variation in resistance at the point (*a*) was eliminated, so that the relations of the resistances at the points (*b*), (*c*) and (*d*) were found for apples of equal resistance at the point (*a*). The partial correlations $r_{bc.a}$ and $r_{cd.a}$ were therefore calculated with the following results :—

$$\left.\begin{array}{l} r_{bc.a} = -0\cdot102 \\ r_{cd.a} = -0\cdot159 \end{array}\right\}.$$

These values are quite insignificant and indicate that in apples of equal resistance at one point no real difference on the average exists between the resistance at any two other points round the circumference. This result was confirmed by taking differences between the figures in columns *b* and *c* and *c* and *d*, and testing by Student's method. The evidence for the specific value of resistance in individual apples is thus seen to be cumulative.

The final test made was to inoculate apples at two opposite points (*a* and *c*). The invasion was allowed to reach a certain stage and then the rotten tissue was removed and weighed, and the holes sealed with wax. The apples were then reinoculated at the alternate points (*b* and *d*) and after a time the amount of rot determined as before. The results are given below in Table V.

Table V.—Radial Advance following two Successive Inoculations of same Individual Apples.

	Radial advance after first inoculation. 16 days.	Radial advance after second inoculation. 10 days.
1	0·47	0·46
2	0·46	0·31
3	0·44	0·30
4	0·40	0·30
5	0·34	0·26
6	0·34	0·29
7	0·34	0·28
8	0·27	0·22
9	0·24	0·21
10	0·24	0·22

A correlation coefficient was determined between the radial advance resulting from the first and second inoculations, respectively. The value found was $r = +0{\cdot}816$, which agrees very well with the values previously determined for correlation in resistance between the halves of single apples. The value has a significance greater than 100 to 1 (6) and finally proves the specific value of resistance in the individual apple.

There still remains the remote possibility that variations in invasion were due to changes in the fungus. To test this, the organism was re-isolated from apples showing extreme variations in attack (100 per cent. and 0·4 per cent. of rot). No marked differences could be detected in the vigour of the fungus as measured by its growth rate on a standard synthetic medium ; and such differences as were found bore *no relation to the amount of rot at the point from which the fungus was re-isolated.*

The results obtained from further applications of the statistical method described in this communication will be presented in the second paper of this series, when certain specific problems connected with the resistance of apples to fungal invasion and the virulence of fungal strains will be considered.

VII.—*Summary.*

1. A standard technique is described for studying the invasion of apples of different varieties inoculated with various species of fungi. The invasion has been studied during various periods of storage life and at different temperatures.

2. The primary data are derived from upwards of 15,000 inoculations and consist of the weights of rotted tissue derived from individual apples. It is shown that, since invasion occurs in a sphere of limited size, a complex relation

holds between the weight of tissue rotted and the penetration of the fungus. In consequence of this the weight of rotted material obtained during two consecutive equal periods of time will not be equal, even though the penetration of the fungus is at a uniform rate. A method has been devised which takes into account this complex relationship, and a new measure "radial advance" is calculated which is constant over equal intervals of time when invasion is proceeding at a uniform rate, irrespective of the stage of invasion reached.

3. The percentage weights of rotted tissue derived from individuals in a sample of apparently uniform apples are found to vary over a wide range. The distribution of percentage weights with a given fungus is shown to be highly asymmetric, while the same data converted into radial advance give a distribution only moderately asymmetric. The standard error of the mean of radial advance is shown to be less than half that of percentage weights, indicating a great gain in accuracy in the use of radial advance as a measure of invasion.

4. The rate of radial advance provides a convenient scale in which to measure the resistance to invasion in individual apples.

5. It is found that each individual apple in a sample has a characteristic resistance. This is shown by:—

(1) The high positive correlation obtaining between the radial advance at the opposite sides of individual apples inoculated at two or four points:—

$$r = +\,0{\cdot}694 \text{ (2 points, 116 apples).}$$
$$r = +\,0{\cdot}742 \text{ (4 points, 20 apples).}$$

(2) By the high correlation found to hold between the radial advances obtained from two successive inoculations of the same individuals by the same fungus:—

$$r = +\,0{\cdot}816 \text{ (10 apples).}$$

LITERATURE CITED.

(1) Kidd, M. N. "The Mortality Curve in a Population of Apples," 'Rep. Food Investigation Board, 1924.' Department of Scientific and Industrial Research, London.

(2) Granger, K., and Horne, A. S. "A Method of Inoculating the Apple," 'Annals of Botany,' vol. 38, p. 213 (1924).

(3) Horne, A. S. "Diagnosis of Fungi from 'Spotted' Apples," 'Journ. of Botany, vol. 58, p. 238 (1920).

(4) Horne, A. S., and Horne, E. V. "Mycological Studies, I. On the 'Spotting' of Apples in Great Britain," 'Annals of Applied Biology,' vol. 12, p. 183 (1920).

(5) Brown, W., and Horne, A. S. "Studies of the Genus Fusarium, I. General Account," 'Annals of Botany,' vol. 38, p. 379 (1924).

(6) Fisher, R. A. "Statistical Methods for Research Workers," Edinburgh, Table V, p. 174 (1925).