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.*

By A. S. HORNE, D.Sc., and F. G. GREGORY, 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 previous paper (1) a method was developed for studying quantitatively the process of invasion of apples by fungi inoculated into them. It was there shown that the method is capable of yielding a measure of the amount of invasion (radial advance) which is independent of the size of the apple used and of the time of duration of the experiment. The distribution of radial advance in populations of apples was studied and it was shown (1) that the distribution of radial advance* (in terms of which resistance to invasion is measured) is not highly asymmetrical if measured at the right stage of invasion, (2) that an individual apple is more uniform with respect to resistance than are any two apples selected at random. It is shown in the course of the present paper that by utilising radial advance as a measure of invasion the change in resistance to the attack of fungal species during the period of storage can be followed. A comparison is made of the virulence of certain fungal species or strains and of the variation in resistance to fungal invasion shown by different populations of given varieties of apples during storage. The significance of these comparisons has been estimated by the calculation of probable errors, which was shown to be

* Resistance has been assumed to be inversely proportional to the rate of radial advance.

legitimate in the previous paper. Finally the bearing of the results obtained on the problem of disease resistance is discussed.

II.—*The Time Sequence of Invasion for Selected Fungal Species.*

Some information had already been obtained in 1921–22 on the behaviour of certain selected fungi. The results attained at that time were based on periodic counts of the number of apples showing externally marked signs of invasion (using samples of 10 apples). In fig. 1 which is a selection of curves
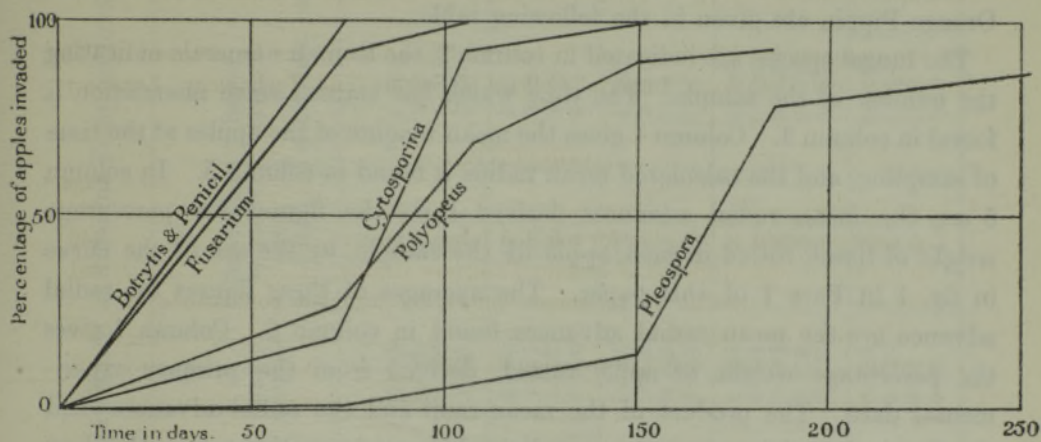

Fig. 1.—Time succession in percentage of apples in a sample showing invasion by various species of fungi. 1° C.

already published (2) the differences in the time of onset of the main invasion are seen to vary greatly for the different species employed, and for this reason some of these species have been selected for further work. As far as the experiment of 1921–22 went it clearly established the fact that the successional invasion of fungal species observed in apples in cold store was not due to a failure of infection, but to a check in development after infection had occurred (3). It seemed desirable, however, to investigate by a more exact method the relation between parasite and host during the period of delay in invasion.

The species selected for further study were (1) *Pleospora pomorum*, (2) *Polyopeus aureus*, (3) *Fusarium* strain A, (4) *Cytosporina ludibunda*, (5) *Botrytis* sp. These were grown on Cox's Orange Pippin apples, obtained from Burwell, Cambridgeshire. One thousand apples were selected and divided into two batches of 500 each. Each batch was inoculated with the five specified fungi (100 apples each). With the first set the inoculations commenced on October 19 and ended on October 23, 1925, using the fungi in the order given above. This set was despatched to the Low Temperature Station, Cambridge, on

October 26 and placed at 12° C. The second set was inoculated exactly one week later, using the same fungi in the same order, and despatched to Cambridge on November 2. These a ples were stored at 3° C.

Each set of 100 apples was divided at random into 5 samples of 20 apples each before despatching the set to Cambridge. Five samples (one for each fungus) were returned to London at more or less regular intervals (fortnightly intervals for those stored at 12° C., monthly intervals for those stored at 3° C.) for systematic examination. The results of the 1925 experiment with Cox's Orange Pippin are given in the following table.

The fungal species are indicated in column 2, the Roman numerals indicating the number of the sample. The time which has elapsed since inoculation is found in column 3. Column 4 gives the mean weights of the apples at the time of sampling, and the calculated mean radius is found in column 5. In column 6 are the mean radial advances derived from the figures for percentage weight of tissue rotted in each apple of the sample, by the use of the curve in fig. 1 in Part I of this paper. The averages of these figures for radial advance are the mean radial advances found in column 6. Column 7 gives the percentage weight of apple rotted, derived from the primary experimental data. The product of the mean radii and the radial advances yield the figures in column 8, and are radial advances in centimetres. Dividing the figures in this column by the number of days elapsed from inoculation the growth rates per day in centimetres, found in column 9, were obtained. The mean growth rates in the intervals between sampling are found in column 10, and were obtained by dividing the increments in radial advance in column 8 by the time elapsed between the two samplings indicated.

The different levels of growth rate exhibited by the various fungi are clearly represented by the figures in columns 9 and 10 of Table I. In the case of *Botrytis*, the growth rate at the higher temperature is approximately double that of the lower, while with *Fusarium* the difference is slightly in favour of the higher temperature. The growth rate of *Polyopeus aureus* at the two temperatures is almost identical. *Pleospora* exhibits a slightly higher growth-rate at the lower temperature and this effect is very marked in the case of *Cytosporina*. With *Fusarium* " interference " occurred at both temperatures.

Averaging the growth-rates at the two temperatures, the order of diminishing values would be *Botrytis*, *Cytosporina*, *Fusarium*, *Polyopeus aureus* and *Pleospora*. This is precisely the order noted in the succession of species already referred to in fig. 1, with the exception of *Fusarium* in which case a weaker strain was utilised for this experiment. The variation in " reaction " exhibited by

Table I.—Rate of Invasion for certain Fungi at different Temperatures.

1. Temperature.	2. Sample.	3. No. of days from inoculation.	4. Mean apple in grams.	5. Mean radius in centimetres.	6. Radial advance.	7. Percentage weight of tissue rotted.	8. Radial advance in centimetres.	9. Growth rate per day in centimetres.	10. Growth rate during interval in centimetres per day.	
°C.										
3	*Botrytis* I	35	63·9	2·63	0·648	24·76	1·704	0·0487	0–I	0·0487
3	,, II	68	65·9	2·65	0·800	36·24	2·12	0·0352	I–II	0·0126
3	,, III	89	54·4	2·49	1·302	91·2	3·24	0·0364	II–III	0·0533
3	,, IV	120	62·5	2·61	1·137	79·8	2·97	0·0248	III–IV	−0·0087
12	*Botrytis* I	19	68·1	2·68	0·850	47·3	2·27	0·119	0–I	0·119
12	,, II	33	67·0	2·67	1·275	89·4	3·40	0·106	I–II	−0·0807
12	,, III	47	67·0	2·67	1·247	86·1	3·33	0·071	II–III	−0·0050
12	,, IV	61	67·0	2·67	1·400	100·0	3·74	0·061	III–IV	0·0293
3	*Cytosporina* I	36	60·69	2·59	0·563	21·0	1·458	0·040	0–I	0·040
3	,, II	70	62·3	2·60	0·816	44·86	2·121	0·030	I–II	0·0195
3	,, III	90	64·3	2·63	0·964	61·0	2·54	0·028	II–III	0·0210
3	,, IV	117	65·7	2·65	1·018	63·8	2·70	0·023	III–IV	0·0059
12	*Cytosporina* I	21	64·8	2·64	0·125	0·9	0·330	0·016	0–I	0·016
12	,, II	35	64·9	2·64	0·164	0·8	0·424	0·012	I–II	0·0067
12	,, III	48	65·5	2·65	0·186	0·9	0·493	0·010	II–III	0·0053
12	,, IV	75	53·9	2·49	0·170	2·0	0·424	0·0057	III–IV	−0·0025
12	,, V	98	57·9	2·56	0·297	11·7	0·760	0·0078	IV–V	0·0146
3	*Fusarium* I	38	68	} 2·71 {	0·322	3·1	0·873	0·023	0–I	0·023
3	,, II	71	68·1		0·376	4·6	1·19	0·015	I–II	0·0096
3	,, III	92	70·9		0·38	4·6	1·19	0·013	II–III	0
3	,, IV	118	71·8		0·38	4·6	1·19	0·010	III–IV	0

Table I—(continued.)

1. Temperature.	2. Sample.	3. No. of days from inoculation.	4. Mean apple in grams.	5. Mean radius in centimetres.	6. Radial advance.	7. Percentage weight of tissue rotted.	8. Radial advance in centimetres.	9. Growth rate per day in centimetres.	10. Growth rate during interval in centimetres per day.	
°C.										
12	*Fusarium* I	22	66·89	2·67	0·336	4·4	0·897	0·0408	0–I	0·0408
12	,, II	36	68·63	2·69	0·378	5·5	1·017	0·0282	I–II	0·0086
12	,, III	49	65·4	2·65	0·296	7·0	1·155	0·0236	II–III	0·0029
12	,, IV	88	62·7	2·46	0·520	22·1	1·279	0·0145	III–IV	0·0032
12	,, V	100	47·7	2·39	0·702	36·0	1·678	0·0168	IV–V	0·0333
3	*Polyopeus* I	38	65·8	2·70	0·235	1·4	0·635	0·0168	0–I	0·0168
3	,, II	71	64·8		0·267	2·6	0·720	0·0102	I–II	0·0026
3	,, III	92	67·0		0·282	4·1	0·762	0·0083	II–III	0·0020
3	,, IV	118	77·8		0·501	21·4	1·328	0·0089	III–IV	0·00217
12	*Polyopeus* I	22	77·7	2·76	0·143	0·6	0·394	0·0179	0–I	0·0179
12	,, II	36	70·7		0·235	1·5	0·648	0·0180	I–II	0·0181
12	,, III	48	72·6		0·252	1·4	0·693	0·0144	II–III	0·0038
12	,, IV	77	60·4		0·252	2·3	0·693	0·0090	III–IV	0
12	,, V	99	53·3		0·329	10·7	0·908	0·0092	IV–V	0·0098
3	*Pleospora* I	40	77·6	2·78	0·225	1·1	0·626	0·0156	0–I	0·0156
3	,, II	74	75·9		0·160	0·7	0·446	0·0064	I–II	−0·0053
3	,, III	94	71·9		0·150	0·7	0·417	0·0043	II–III	−0·0015
3	,, IV	120	64·6		0·196	1·1	0·547	0·0046	III–IV	0·0050
12	*Pleospora* I	24	67·2	2·69	0·104	0·4	0·280	0·0117	0–I	0·0117
12	,, II	38	69·3		0·218	1·2	0·586	0·0154	I–II	0·0218
12	,, III	51	63·7		0·185	1·5	0·342	0·0067	II–III	−0·0187
12	,, IV	79	61·9		0·191	2·9	0·514	0·0065	III–IV	−0·0061
12	,, V	101	58·3		0·262	3·5	0·705	0·0070	IV–V	0·0086

the *Fusarium* strains is dealt with later. In every case the rate of growth falls in the early part of the experiment, but in the case of *Fusarium* at 12° C. and of *Pleospora* at 12° C. there is an indication of a subsequent rise.

In spite of the part played by sampling errors there is an unmistakable similarity in the drift of growth-rate. The growth-rate falls rapidly in each case until the neighbourhood of the fiftieth day where a minimum occurs, and subsequently in most cases there is an increase in growth-rate. Averaging the values* in all the experiments the following figures are obtained.

Table II.—Mean Values for Growth-rate in time for the Fungi in Table I.

Days from inoculation.	Mean growth-rate in centimetres per day.
20	0·031
30	0·022
40	0·0086
50	0·0072
60	0·0074
80	0·013

The growth-rates have been assumed to be inversely proportional to the resistance of the apple to invasion, and since the growth-rates of all the fungal species employed exhibit the same kind of time-relation, irrespective of temperature, there is possibly a general change in resistance occurring in every case. It is probable that the resistance of the apples was increasing up to the fiftieth day from inoculation, with slight indication of a subsequent fall. If it is assumed that the variation in resistance with time is represented by a curve of the kind shown in fig. 2 and that L 1 and L 2 represent two critical levels of resistance for the two species of fungus F 1 and F 2, then for species F 1 the growth-rate will fall during the interval of time from 0 to T 1 (since during this time the resistance is rising) will reach a minimum somewhere between T 1 and T 2, and will subsequently rise as the resistance once again falls. Such a distribution of growth-rates is seen in the case of *Botrytis* at 3° C. In the case of species F 2 the growth-rate will fall rapidly up to a time T 1 at which point the resistance rises above the critical level. Growth will then cease until such time as the resistance again falls below the critical value. If the portion of the apple which has been already invaded before the first critical point is reached is not negligibly small when growth is checked, subsequent drying out may lead to development of cavities which are characteristic of the phenomenon of " interference." This effect

* The values on the particular days were obtained by interpolation from curves.

was very evident with *Fusarium* at both temperatures and sporadically with
Polyopeus aureus. In the case of slow-growing fungi the volume of the apple
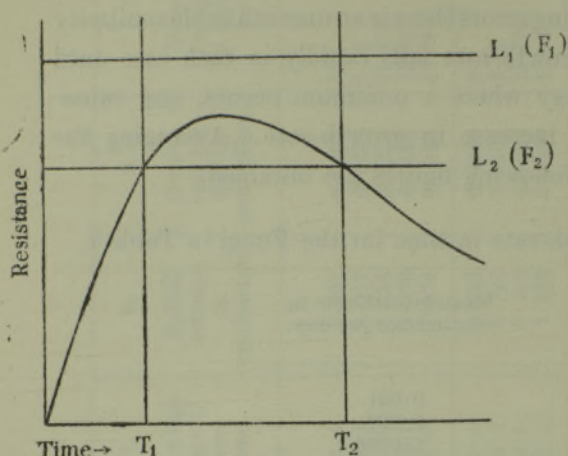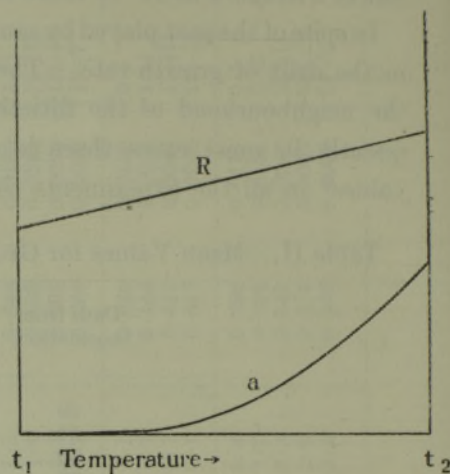

FIG. 2.

FIG. 3.

affected before the growth ceases would be so small that the cavities would not
be evident.

The varying reactions of the fungi at the two temperatures is striking and is
difficult to interpret. The relation between parasite and host will depend on
the effect of temperature, on the activity of the fungus, and the resistance of
the apple. For *Botrytis*, which is a virulent species, the activity is found to
increase to double for the temperature rise of 9°, which is near the normal
temperature coefficient of growth. In the case of *Fusarium* and the other fungi,
the increase in activity is not evident. It is possible that it is masked by the
relatively higher resistance of the apple in these cases.

Should the temperature coefficients of resistance and activity be greatly
different, the temperature relations may be formally represented by the diagram
shown in fig. 3. The rate of invasion has been assumed to be proportional to
$1/R$, where R is the resistance of the apple. If a is the activity of the fungus
we may write

$$V = \frac{a}{R + C}$$

where V is the rate of radial advance and C a constant. It is clear that if the
relations indicated in the diagrams hold, $a/R + C$ may have a minimum value
between temperatures T 1 and T 2. Such a set of relationships might account
for the results obtained with *Cytosporina* at 12° C. and 3° C. ; and by suitable

modification of the temperature effect on R, such cases as *Fusarium* and *Polyopeus aureus* would be explicable. With our present knowledge it is impossible either to substantiate or refute such a hypothesis, but further lines of work are clearly indicated. The evidence to hand as to the nature of resistance will be discussed later in this paper.

III.—*Comparison of the Virulence of Certain Fusarium Strains.*

An interesting aspect of the problem of invasion of apples is the relative degree of virulence of various strains of a single species of fungus. In the preceding section of this paper different species have been compared, and similar methods will now be applied to various strains of a given species. As some of the strains studied are saltant forms whose origin and previous history are well known, it has been possible to compare these with their parent strains. The practical importance of such comparison in relation to the possible origin of new diseases of apples is evident.

The *Fusarium* strains used for these experiments were originally isolated from diseased apples. The strain A was obtained by Dr. W. Brown of this College, in 1918, from an unknown variety ; the strains B, C, and D from Cox's Orange Pippin, E from Allington Pippin, and F from Sweet Alford, in 1920 by one of the present authors (A.S.H.). The strains B 1, C 1 and C 2 originated from monosporous platings of B and C respectively, and were made in 1921. C 3 was first obtained as a variant from C in tube cultures made from the same monosporous colony of C. The strains B 11, B 111 and D 2 are saltants obtained by Brown, who has recently dealt with the question of saltation in some detail (4). The origin of the strains and saltants is shown diagrammatically below (fig. 4).

The fact that this species of *Fusarium* was capable of parasitising the tissues of the apple was first shown in 1921 (3, p. 369) when, with the exception of *Penicillium* and *Botrytis*, it proved at 1° C. the most active fungus tested in that year (see fig. 1). In 1922 eight strains were tested on the Cox's Orange Pippin and Bramley's Seedling varieties of apples at two temperatures (1° C. and 15° C.). The strains included A, C, C 1, C 2, B 1, D, E, and F. All the strains employed invaded both the varieties at 15° C., but the intensity of attack was much less marked in the case of the Bramley's. At 1° C., the Cox's were slightly invaded by the strains C, C 1, C 2, F, D, B 1, and E. The invasion of the Bramley's at this temperature was negligible (5, p. 39, and 6, p. 59).

As a result of experiments begun in 1923 using the strains C 1, C 2 1, C 3, B 1, B 1 1, A and D on Cox's Orange Pippin at two different temperatures (12° C. and 3° C.) not only was the parasitic activity of these *Fusarium* strains con-

Date.	Origin.	Original strain.	Saltants.
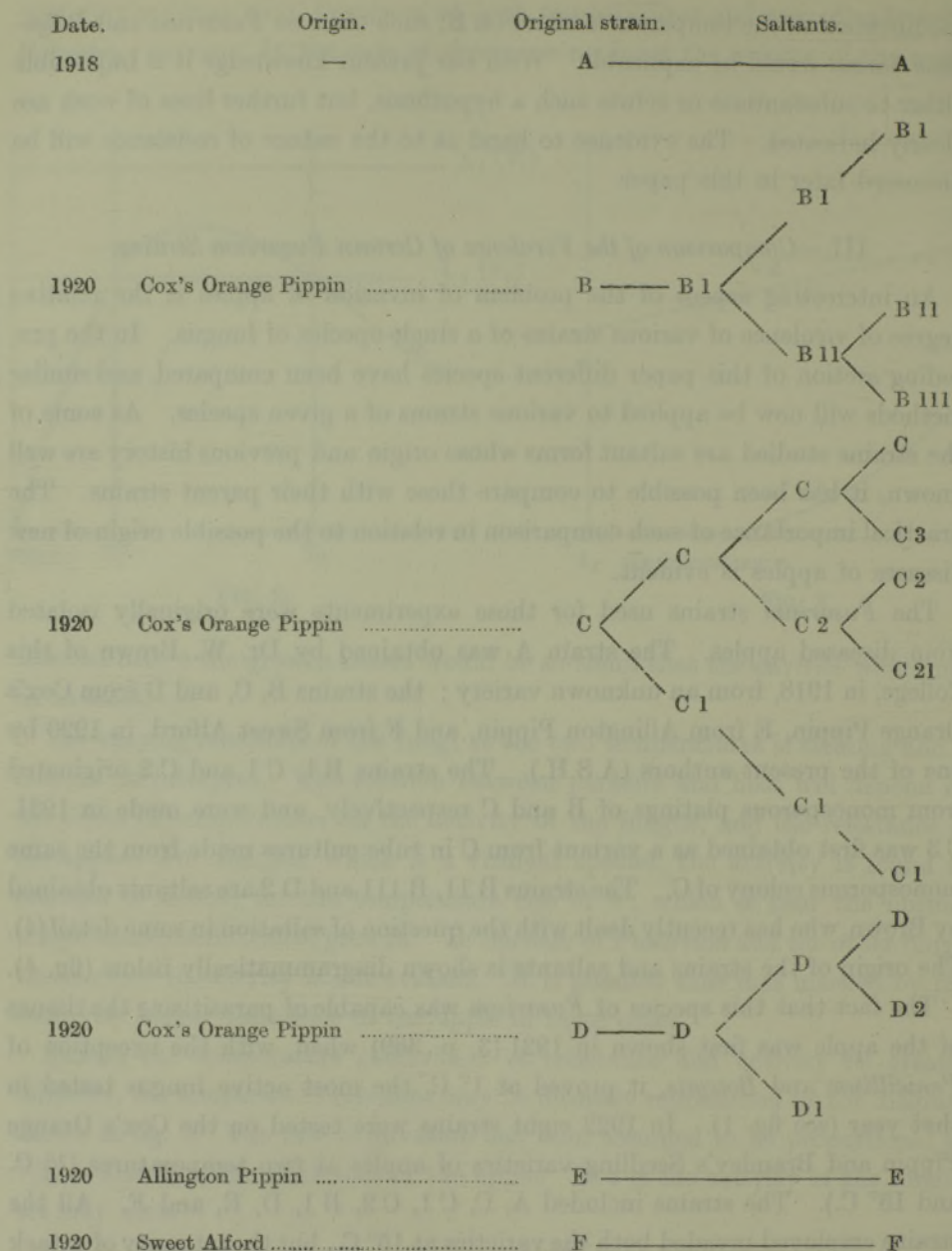
FIG. 4.—Pedigree of *Fusarium* strains.

firmed, but in addition it was clearly evident that they varied in their pathogenicity (6, p. 62). Certain strains such as D 1, C 1, C 21, and B 1 being very active, others (A, C 3) moderately active and others (B 11) very weak. It was found that the strong strains belonged to the group of strains which exhibit

strong mycelial development, whereas the weak strains were those characterised by feeble mycelial development and intense sporulation (see (7)). It was also clear that the saltant B 11 derived from the strongly active strain B 1 differed from the parent in a marked loss of virulence.

In 1924 and 1925 the same experiments were repeated on a larger scale, with Bramley's in addition to the Cox's, using the more exact methods for recording data already outlined in this paper. The results obtained in both years confirm the earlier work, and evidence will be presented to show that the strains can be arranged in serial order with respect to their pathogenicity, and that the sequence is independent of the type of apple used.

The data obtained are summarised in Table III in which the rate of advance in centimetres per day is given in column 6 for each of the strains of *Fusarium* given in column 1. The probable errors for the rates of advance have been calculated and the significance of the differences between strains can thus be tested.

Table III.—Rates of Invasion for certain *Fusarium* Strains in two Varieties of Apple.

1.	2.	3.	4.	5.	6.
Fusarium strain.	Mean weight of apple in grams.	Mean radius in centimetres.	Radial advance.	Radial advance in centimetres.	Rate of advance per day in centimetres.
Bramley's Seedling, 1925–26. 12° C. 70 days.					
A	156·2	3·66	0·557 ± 0·030	2·04 ± 0·15	0·0292 ± 0·0021
B 11	153·9	3·65	0·337 ± 0·017	1·23 ± 0·065	0·0178 ± 0·0009
B 111	137·8	3·51	0·356 ± 0·021	1·25 ± 0·077	0·0179 ± 0·0010
C 1	141·0	3·53	0·469 ± 0·042	1·66 ± 0·15	0·0237 ± 0·0021
C 21	145·8	3·58	0·486 ± 0·043	1·74 ± 0·17	0·0248 ± 0·0024
C 3	147·8	3·60	0·523 ± 0·046	1·88 ± 0·17	0·0296 ± 0·0024
D	174·9	3·81	0·595 ± 0·036	2·26 ± 0·14	0·0322 ± 0·0020
D 2	145·3	3·58	0·367 ± 0·018	1·31 ± 0·068	0·0188 ± 0·0010
		Mean P.E. ± 0·056			
Bramley's Seedling, 1924–25. 12° C. 103 days.					
A	91·3	3·07	0·378 ± 0·041	1·160 ± 0·13	0·0113 ± 0·0013
B 1	90·9	3·06	0·606 ± 0·038	1·854 ± 0·13	0·0180 ± 0·0013
B 11	115·5	3·05	0·414 ± 0·043	1·263 ± 0·13	0·0123 ± 0·0013
B 111	99·5	3·15	0·131 ± 0·027	0·413 ± 0·11	0·0040 ± 0·0011
C 1	101·2	3·17	0·754 ± 0·054	2·39 ± 0·17	0·0232 ± 0·0017
C 21	106·8	3·23	0·622 ± 0·037	2·009 ± 0·12	0·0195 ± 0·0012
C 3	88·1	3·03	0·652 ± 0·048	1·976 ± 0·15	0·0192 ± 0·0015
D	100·2	3·16	0·714 ± 0·046	2·256 ± p·15	0·0219 ± 0·0015
		Mean P.E. ± 0·048			

Table III—(continued).

1. Fusarium strain.	2. Mean weight of apple in grams.	3. Mean radius in centimetres.	4. Radial advance.	5. Radial advance in centimetres.	6. Rate of advance per day in centimetres.
Cox's Orange Pippin, 1924–25. 12° C. 54 days.					
A	66·54	2·66	0·860 ±0·062	2·288 ±0·166	0·0424 ±0·0031
B 1	66·13	2·66	0·952 ±0·080	2·532 ±0·214	0·0469 ±0·0040
B 11	67·59	2·68	0·380 ±0·037	1·018 ±0·099	0·0188 ±0·0018
B 111	64·77	2·64	0·103 ±0·023	0·272 ±0·061	0·0050 ±0·0011
C 1	59·33	2·57	1·025 ±0·063	2·634 ±0·264	0·0487 ±0·0030
C 21	70·06	2·71	0·953 ±0·024	2·583 ±0·065	0·0478 ±0·0012
C 3	68·11	2·69	0·786 ±0·068	2·114 ±0·184	0·0392 ±0·0034
D	64·01	2·63	1·038 ±0·049	2·730 ±0·132	0·0506 ±0·0025
		Mean P.E. ±0·027			
Cox's Orange Pippin, 1924–25. 3° C. (corrected for interference). 138 days.					
A	68·7	2·74	0·531 ±0·046	1·455 ±0·128	0·0105 ±0·00093
B 1	61·7	2·64	0·747 ±0·069	1·972 ±0·184	0·0143 ±0·0010
B 11	66·7	2·71	0·194 ±0·043	0·526 ±0·117	0·00381 ±0·00085
B 111	72·4	2·78	0·016 ±0·011	0·046 ±0·031	0·00033 ±0·00023
C 1	68·6	2·74	0·618 ±0·061	1·693 ±0·169	0·0122 ±0·0012
C 21	68·0	2·73	0·587 ±0·062	1·603 ±0·171	0·0116 ±0·0013
C 3	62·2	2·65	0·813 ±0·054	2·154 ±0·142	0·0156 ±0·0010
D	73·1	2·79	0·598 ±0·077	1·668 ±0·216	0·0121 ±0·0016
		Mean P.E. ±0·036			
Cox's Orange Pippin, 1925–26. 12° C. 89 days.					
A	62·7	2·46	0·541 ±0·044	1·33 ±0·109	0·015 ±0·0012
B 11	60·6	2·44	0·240 ±0·020	0·59 ±0·049	0·0066 ±0·00055
B 111	67·8	2·53	0·239 ±0·0088	0·60 ±0·024	0·0067 ±0·00027
C 1	64·4	2·44	0·800 ±0·023	1·95 ±0·101	0·022 ±0·0011
C 21	54·5	2·35	0·750 ±0·072	1·76 ±0·187	0·020 ±0·0021
C 3	57·4	2·40	0·520 ±0·075	1·25 ±0·181	0·014 ±0·0020
D	60·9	2·44	0·840 ±0·023	2·05 ±0·062	0·023 ±0·00069
D 2	63·1	2·47	0·353 ±0·052	0·87 ±0·129	0·0098 ±0·0014
		Mean P.E. ±0·033			

The data of rate of advance have been presented again in serial order in Table IV, the most rapidly growing strain being in each case in the first column.

From Table IV it is clear that the two experiments with Cox's Orange Pippin agree exactly in the order of virulence of the strains, when D 2 and B 1 which were not common to the two experiments are left out of account. This agree-

ment is the more striking when it is observed that the growth-rate in the 1925–26 set is very much less than for 1924–25.

Table IV.—Rates of Invasion for the same *Fusarium* Strains arranged in serial order.

	Strains of *Fusarium* and radial advance in centimetres per day.							
Bramley's Seedling, 1925–26. 12° C. 70 days.	D 0·0322 ±0·0020	C 3 0·0296 ±0·0024	A 0·0292 ±0·0021	C 21 0·0248 ±0·0024	C 1 0·0237 ±0·0021	D 2 0·0188 ±0·0010	B 111 0·0179 ±0·0070	B 11 0·0178 ±0·0009
Bramley's Seedling, 1924–25. 12° C. 103 days.	C 1 0·0232 ±0·0017	D 0·0219 ±0·0015	C 21 0·0195 ±0·0012	C 3 0·0192 ±0·0015	B 1 0·0180 ±0·0013	B 11 0·0123 ±0·0013	A 0·0113 ±0·0013	B 111 0·0040 ±0·0011
Cox's Orange Pippin, 1925–26. 12° C. 89 days.	D 0·023 ±0·00069	C 1 0·022 ±0·0011	C 21 0·020 ±0·0021	A 0·015 ±0·0012	C 3 0·014 ±0·0020	D 21 0·0098 ±0·0014	B 111 0·0067 ±0·00027	B 11 0·0066 ±0·00055
Cox's Orange Pippin, 1924–25. 12° C. 54 days.	D 0·0506 ±0·0025	C 1 0·0487 ±0·0030	C 21 0·0478 ±0·0012	B 1 0·0469 ±0·0040	A 0·0424 ±0·0031	C 3 0·0392 ±0·0034	B 11 0·0188 ±0·0018	B 111 0·0050 ±0·0011
Cox's Orange Pippin, 1924–25. 3° C. 138 days.	C 3 0·0156 ±0·0010	B 1 0·0143 ±0·0010	C 1 0·0122 ±0·0012	D 0·0121 ±0·0016	C21 0·0116 ±0·0013	A 0·0105 ±0·00093	B 11 0·0038 ±0·00085	B 111 0·00033 ±0·00023

The agreement between the corresponding Bramley's series in relation to order of virulence is much less marked. For instance C 1 in 1924–25 occupied the first place while in 1925–26 it was fifth in order. Strain A also has changed from being the last but one in 1924–25 to the third place in 1925–26. Similar discrepancies are seen in the set of *Fusarium* strains growing in Cox's Orange Pippin at 3° C. as compared with those at 12° C.

The records made at the time of estimating the rot show that signs of withering were noted in both the sets with Bramley's Seedling and the Cox's at 3° C. In the case of the Bramley's 1924–25 set and the Cox's 3° C., notes with regard to withering were made on each individual apple, and it was possible in these cases to arrive at an estimate by noting the proportion of apples which showed signs of withering. As it seemed possible that the withering had partly accounted for the changed order in these cases, as compared with the Cox's at 12° C., correlations were worked out in each case between the percentage of withered apples in a set and the growth-rates. The results gave for Bramley's 1924–25, $r = -0.833$ and for Cox's $r = -0.666$. The negative sign in both cases indicates that the growth-rate tends to fall with increasing intensity of withering.

As it was thought that these correlations may have been due to a spurious

effect—since in general the greatest withering occurred with the slowest growing strains, and the possibility of the absorption of water by the living tissue from the dead watery areas could not be neglected—another method of correlation was resorted to. In this case the rates of advance calculated for the set of Bramley's 1925–26 where withering was not so marked, and the set of Cox's 1924–25, 12° C., where withering was not noted, were taken as normal. The ratios of the growth-rates of these sets as compared with corresponding abnormal sets were determined and their ratios correlated with the observed percentage of withering. The correlation coefficients were for Cox's $r = + 0 \cdot 738$ and for the Bramley's $+ 0 \cdot 789$. The positive sign of this result indicates that where the percentage of withering was high, the ratio of the rate of growth in the normal set as compared with the abnormal set tended to rise ; or, in other words, in the withered apples the growth-rate of the fungus was suppressed.

As in this method the rates in apples affected with the same strain were compared, there is no question of a differential effect on water loss due to the presence of different amounts of rotten tissue. The evidence seems to point to a real effect of withering on the growth-rate of the fungus, and may possibly arise by competition for water between the fungus and the host tissues, or an increase in concentration of the cell contents. The drying-out of the rotten areas, in cases where "interference" occurs, indicates that absorption of water from the dead tissues can actually occur, and experiments to test the effect of water loss are now in progress.

In order to assess the relative effects of apple variety, of season, and of temperature on the rate of invasion by different strains of *Fusarium*, Fisher's method of analysis of variance (8) was applied to the data. It was assumed that the rate of radial advance is determined by the interaction of two factors, viz., the susceptibility of the apple and the activity of the strains, and also that the rate of advance was proportional to the products of these two factors. For this reason in the process of analysis the logarithms of the values for radial advance were used instead of the actual values recorded. Seven strains were investigated, D 2 and B 1 being omitted since the records for these two strains were not complete. Variety of apple and experimental conditions (season and temperature) yielded five combinations. Thus there were in all 35 combinations of experimental conditions with fungal strains giving 34 degrees of freedom, 7 fungal strains with 6 degrees of freedom, and 5 experimental conditions with 4 degrees of freedom, respectively. The results of the analysis are shown in Table V.

Table V.—Analysis of Variants.

	Degrees of freedom.	Variance.	Mean square.
Strains	6	2·68179	0·44699
E. conditions	4	1·40910	0·35228
Remainder	24	1·61746	0·06740
Total	34	5·70835	—

The 24 remainders give an estimate of the mean discrepancies of individual results from the assumed product formula. The figure corresponds to a standard error of 16·7 per cent. for a single determination. From the figures for probable error of radial advance in Table III it is possible to compare this calculated mean error with the experimental errors actually recorded, and thus to test whether the departures from the product formula were significant. For strain D the mean error recorded in the experiments was 11·4 per cent., for strain C 3 14·0 per cent., while for strain B 111, in which variation in radial advance was greatest, the value of the mean error was 29·7 per cent.

It is clear then that the errors recorded in the experimental data agree very well with the expected discrepancy from values for radial advance based on the assumption that this process is conditioned by the product of activity of fungal strains and susceptibility of apples—susceptibility is taken to be the reciprocal of resistance.

Table V shows further that the differences in strains had the largest effect on rate of advance, but that the experimental conditions were almost as effective. There is no significant difference between these two effects, but both show very high significance (about eight times their mean error) as compared with the random errors.

The effect of experimental conditions can be traced almost entirely to the difference between the experiments at 12° C. and the single experiment at 3° C. The seasons and variety of apple show no significant differences in their effects among themselves. It may be concluded from this that the variety of apple and seasonal effects do not greatly affect the relative virulence of the different strains tested, while there are indications that at different temperatures the amount of infection due to different strains relative to each other may vary. The effect of different levels of temperature on the relative virulence of fungal species has already been observed, and it appears that similar relations may hold for strains of the same species.

This analysis of variance indicates further that in general the effect of the

various fungal strains is due largely to the two strains B 11, B 111, which differ significantly from the rest, while in the data no significant difference among the others is found. Taking the rate of radial advance of the highest strain as 100 and calculating the others as a percentage for each set, the averages for each strain based on all the data are given in the following table (Table VI).

Table VI.—Comparison of Rates of Radial Advance for *Fusarium* Strains.

Strain.	Relative figure of radial advance.	Strain.	Difference in radial advance.
D	94·4±3·3		
C 1	88·7±3·3	D—C 1	5·7±4·7
B 1	87·5±4·6	D—B 1	6·9±5·7
C 21	83·4±3·9	D—C 21	11·0±5·1
C 3	82·6±3·7	D—C 3	11·8±5·0
A	72·1±3·1	D—A	22·3±4·5
D 2	50·5±4·3	D—D 2	43·9±5·4
B 11	39·7±2·6	D—B 11	54·7±4·2
B 111	22·7±1·5	D—B 111	71·7±3·6

The probable errors for each value are also given, together with the probable error of the difference between the rates of advance of the different strains as compared with strain D.

If three times the probable error is taken as significant it is seen that strains D, C 1, B 1, C 21, and C 3 do not differ significantly, while the rest, viz., A, D 2, B 11 and B 111, fall into a separate class. Furthermore, B 11 and B 111 differ significantly from strains A and D 2 and also *inter se.* The strains may then be divided into three groups D to C 3 of high, and A and D 2, medium, and B 1 and B 111 low virulence.

The history of the B strains is very interesting, for with each saltation the virulence is seen to diminish. The same relation holds for D and D 2 and also C 1, C 21 and C 3. In the last case cited the difference in virulence is not high enough to be significant, but the fact that this order is identical in the two experiments with Cox's at 12° C., as well as for the average of all, indicates strongly that, in fact, the virulence of these three strains falls into the order given. In the case of the D strains, the saltant is found in the medium class, while for the B strains in spite of the fact that B 1 is in the highest class, both saltants fall into the lowest class.

A similar relation has been found to hold with certain strains of the section *Discolor* of *Fusarium,* recently investigated in this laboratory by Horne and Mitter (9). It is perhaps significant that, in all cases of saltation whose history

is known, the parent form displays a higher virulence than the strains derived from it, although the fall in virulence may vary greatly in magnitude.

IV.—*Comparison of Variation in Resistance shown by different Populations of Bramley's Seedling Apples.*

Experiments were started in the season 1922–23 to investigate the resistance of apples derived from different localities in relation to a particular fungal species. The variety of apple chosen was Bramley's Seedling and the fungus was *Cytosporina ludibunda.* The apples were obtained from two localities, viz., Burwell, Cambridgeshire (fen soil) and Spalding (silt soil). Batches of 100 apples each were inoculated and stored at Cambridge under constant conditions of humidity and temperature (1° C.). After some months the apples were examined and separated into the classes invaded and not invaded. Since " internal breakdown " (a functional disease encountered when apples are stored under low-temperature conditions) was present, the number showing signs of this trouble was also recorded. The result is given in the following table (Table VII).

Table VII.—Effect of Locality of Origin of Apples on Invasion, 1922–1923.

Locality.	Percentage of apples invaded by *Cytosporina.*	Percentage of apples invaded by *Cytosporina* and affected with internal breakdown.	Percentage of apples with internal breakdown.	Percentage of apples not invaded.	Interval in days.
Burwell (fen)	0	15	77	8	177
Spalding (silt)	32	18	10	40	237

It will be seen that the two categories of Bramley's yielded different results. Thus only 15 of the Burwell apples were invaded, whereas 50 of the Spalding apples showed signs of invasion. These figures are misleading, since of the fen apples which were affected a large percentage gave large amounts of rot, while silt apples showed very small amounts, and indeed all showed " interference." The need for an improvement on the method of assessing the resistance by the mere counting of numbers invaded became obvious at once and led to the development of the technique described in the previous paper (1).

In the following year, 1923–24, apples from six localities were obtained and

stored after inoculation at 3° C. In addition to noting those apples which showed no apparent invasion and those completely rotten, the number in the intermediate class was recorded and measurements were made of the rotten areas. The results as obtained in 1923–24 are recorded in Table VIII.

Table VIII.—Effect of Locality of Origin of Apples on Invasion, 1923–1924.

Locality.	Percentage completely rotten.	Percentage intermediate stages.	Percentage not invaded.	Percentage with internal breakdown.	Interval in days.
Spalding (silt)	3	18	72	98	164
Wisbech (fen)........	3	59	38	—	140
Worcester (old red sandstone)	10	84	6	—	120
Canterbury (gravel)	36	56	8	—	118
Burwell (chalk)	45	55	0	—	122
Burwell (fen)	62	38	0	—	116

Before the new measure (radial advance) could be utilised for this set of data, it was necessary to be able to convert the measurements of area into radial advance. This was done by means of two sets of data collected in the years 1924–25 and 1925–26, relating to Bramley's Seedling from Canterbury and from Burwell, for which both measurements of area and the weights of rotten tissue were recorded ; 137 measurements of area and weight records were obtained. The percentage weights of rotten tissue were converted into radial advances from the graph (fig. 1) given in the previous paper (1), and a correlation diagram was set up showing the relation between radial advance (measured as percentage of radius) and the mean diametral arcs of the areas. The regression line was found to be linear, and to be practically identical for the figures derived from the two sets of apples. The correlation coefficient estimated from all the figures was $r = + 0 \cdot 924$. From the regression line the percentage radial advance corresponding to any given area could be read off. In this way the data for areas measured in 1923–24 were converted into radial advances, and the results obtained for the apples from the six localities are given in Table IX.

The figures show large differences in the rate of radial advance for apples from different localities, and this confirms the empirical results already presented in Table VIII. Chemical analysis of samples of apples from the same localities gathered at the same time are available and have been published by H. K. Archbold (10) and Janet W. Brown (11). It is therefore possible to investigate the question as to whether chemical composition plays any part in deter-

Table IX.—Data of Invasion in Table VII in terms of Radial Advance.

Locality and soil.	Percentage radial advance.	Average percentage weight rotten.	Days.	Radial advance centimetres per day.
Spalding (silt)	0·0498	< 1·0	164	0·0010
Wisbech (fen)	0·336	3·5	140	0·0084
Worcester (old red sandstone)	0·722	27	120	0·0201
Canterbury (gravel)	0·832	39	118	0·0247
Burwell (chalk)	0·977	60	122	0·0277
Burwell (fen)	1·104	87	116	0·0333

mining the relative rate of advance in the sets of apples. The rate of advance was first correlated with the nitrogen and potassium content, using the fresh weight figures in the papers already referred to. The result is as follows :—

$$r_{RN} = + 0·7622 \qquad R = \text{radial advance per day.}$$
$$r_{RK} = - 0·3951 \qquad K = \text{percentage K in fresh weight of apples.}$$
$$r_{NK} = - 0·2896 \qquad N = \text{percentage N in fresh weight of apples.}$$

There was found to be no correlation with the phosphate content of the apples.

The negative correlation between nitrogen and potassium in the series of apples used indicated the necessity for eliminating the effects of the association of these two factors in determining the correlation of each with radial advance. The partial correlations are shown below :—

$$r_{RN.K} = + 0·737 \qquad r_{RK.N} = - 0·282.$$

These correlations show that when the effect of the negative correlation of radial advance with potassium content has been allowed for, a large positive correlation of radial advance with nitrogen content is found, while the correlation with potassium is slightly negative, when the effect of nitrogen is eliminated. The high positive correlation of the rate of advance with nitrogen is suggestive, but not significant, since only six pairs of values were available. The negative correlation with potassium is not significant. The results indicate that high nitrogen content of apples enhances the susceptibility to the fungus used, while high potash content tends to increase resistance. The need for further investigation along these lines is evident and further work is now in progress.

V.—*Concerning Resistance to Fungal Invasion in Apples.*

Reviewing the results presented in this and the former communication it is seen that (1) the resistance of the individual apple is of a specific nature ;

(2) apples of a given variety differ in their resistance to different species of fungus and strain of a given fungus ; (3) variation in resistance of a given type of apple is associated with the season in which the apples are grown.

From a consideration of these facts, it seems reasonable to suppose that resistance is of a complex nature. It has been shown that resistance may be correlated with chemical composition of apples, and evidence has been presented which indicates that water content also plays a part.

The differences in resistance of varieties can to some extent be correlated with their known differences, such as variation in acidity, the percentage and nature of the sugars, variation in pectic content, and so on. Preliminary experiments to ascertain the effect of acidity have been carried out by growing various species of fungi in extracts of known pH value from apples of different varieties. The results, although merely qualitative, do show differences between the behaviour of different fungi when grown in extracts of known acidity. Two experiments were made, one with apple extracts which were made at the time of picking, and a second approximately six months later, with apples from the same original batch which had been kept at 1° C. The result of the first experiment has been published elsewhere (2, p. 50, Table I).

From this experiment it appeared that *Penicillium* and *Botrytis* grew in all the extracts, which agrees well with their known behaviour on apple varieties, while *Cytosporina*, *Fusarium* and *Pleospora* did not grow in any of the Bramley's Seedling extracts, although their behaviour differed among themselves in the extracts from the other varieties. The case of *Cytosporina* is interesting, in that growth was found in some of the Bramley's extracts made after six months' storage, when the pH was found to have fallen, which indicates the possibility of changes in resistance due to some tissue change in the apple. The experiments also indicated that there is a limiting level of acidity for particular species beyond which growth cannot proceed.

The relation of the growth of *Pleospora pomorum* in apple extracts of increasing acidity is brought out well by the experimental data obtained in 1921 with extracts of apples kept at 20° C. (3, p. 371). The results show increasing inhibition of growth with increasing acidity.

Further evidence indicating the same effect was obtained by inoculating apple varieties of known pH value and noting which varieties were infected with *Pleospora*. Sweet Alford, pH 4·1 — 4·6, Dymock Red, pH 3·9 — 4·0, Allington Pippin, pH 3·05 — 3·35, were invaded, whilst Bramley's Seedling, pH 3 — 3·1, was unaffected (see 3, p. 371).

The behaviour of *Pleospora* growing in extracts—(see 7, p. 50, Table I)—

differs from the behaviour of the fungus when inoculated into apples, in that under the latter circumstances no growth is found with Cox's Orange Pippin until the expiry of a considerable period of time (see fig. 8). This may perhaps be related to the ascertained fact that *Pleospora* is practically incapable of inverting cane sugar. The following table (Table X) shows the differences of growth of *Fusarium, Pleospora,* and *Cytosporina,* in liquid nutrient media containing various sugars as the source of carbon. Figures for the amount of sugar destroyed and amount of cane sugar inverted are also given.

Table X.—Effect of Sugars on Growth of certain Fungi in Culture Solution.

Fungal species.	Solution.	Weight of mycelium.	Sugar inverted.	Sugar destroyed.
		gms.	mgms.	mgms.
Fusarium	Sucrose + nutrient	0·1187 ⎱ 0·1532 ⎰	340	45
	Glucose + nutrient	0·1041 ⎱ 0·1177 ⎰	—	358
	Fructose + nutrient	0·0401 ⎱ 0·0393 ⎰	—	156
Pleospora pomorum	Sucrose + nutrient	0·0085 ⎱ 0·0108 ⎰	16·8	9·5
	Glucose + nutrient	0·0552 ⎱ 0·0436 ⎰	—	125
	Fructose + nutrient	0·0190	—	99
Cytosporina ludibunda	Sucrose + nutrient	0·2148	271·0	33·6
	Glucose + nutrient	0·2083	—	935

The differences between *Fusarium, Cytosporina* and *Pleospora* with respect to the inversion of cane sugar are very striking, as is also the different amount of growth made by the three fungi in a cane sugar medium. From such experimental evidence, the importance of the nature of the sugars present and the changes which these sugars undergo cannot be overlooked. It seems possible that the growth of *Pleospora* in the extracts derived from Cox's Orange Pippin in the table previously published ((2), p. 50, Table I) may have been due to inversion of cane sugar during the process of sterilising the medium.

The evidence taken altogether suggests very strongly that the immediate cause of resistance in different varieties of apple may vary ; the resistance, for instance, shown by Bramley's Seedling may be due largely to high acidity, while the resistance of Cox's Orange Pippin may be due to the fact that in these apples the ratio of cane to invert sugar is higher than for Bramley's, or to other chemical factors. Indeed the resistance of any one variety, or any

individual apple, will be determined by the interaction of all the known factors, such as, (1) acidity, (2) the nature of the sugars, (3) the percentage of nitrogen, (4) the percentage of potash, (5) the rate of loss of water, as well as possible other factors at present unknown. In so far as the intensity of these factors changes during the period of storage the resistance of individual apples to individual fungi will also show changes.

That such changes do occur is indicated strongly by the data for growth in Cox's Orange Pippin (Table I). The precise formulation of the factor-changes is at present not possible, and awaits more closely correlated data of invasion of apples by specific fungi and concurrent chemical analysis made at various times during storage ; an investigation is proceeding along these lines. With our present knowledge it may be stated, as a well ascertained fact, that the acidity falls during the period of storage, so that the time of onset of invasion for a fungus intolerant of acid may be delayed until a critical level of acidity has been passed. Such a case seems to be indicated by the behaviour of *Cytosporina*, where growth did not occur in extracts from Bramley's Seedling apples until the fruit had been stored for a period of six months. Even when invasion occurs early in the period of storage, in Bramley's Seedling, which is characterised by high acidity, the decreasing acid content and consequent lowering of resistance will be reflected in an increasing rate of radial advance.

Experiments now in progress show conclusively that for apples inoculated with *Cytosporina ludibunda* and kept at 20° C. such a difference of resistance exists between those picked early and those picked late in the season. This fall of resistance in the case of Bramley's Seedling contrasts with the indicated rise in resistance for the Cox's Orange Pippin shown in Table II. Early and late picking is found in the case of Cox's to lead to a difference of resistance against the invasion of *Fusarium* and *Pleospora*, but in this case the later picked apples are found to have *a higher resistance*, which is in accordance with the estimate of change in resistance found by the measurements of radial advance. Evidence may be cited for the increase in resistance with the date of picking from early data obtained with *Pleospora pomorum* in 1922–23 (see Table XI).

Table XI.—Effect of Date of Gathering on Invasion by *Pleospora pomorum*.

Date of gathering.	Per cent. invaded.	Days in store.
October 2	69	168
October 26	20	155

The interaction of two such factors as water content and acidity—the high values of the first favouring invasion, and of the second inhibiting invasion ; both factors, however, changing with time, may lead to those postulated changes in resistance which were schematically presented in fig. 2, in the attempt to account for the phenomenon of "interference."

It may be claimed for the method adopted in this paper that considerable light has been thrown on the problem of resistance of apples to the invasion of fungi. The difficulty of the problem is apparent, but not insuperable ; new lines of work have been opened up, and are being actively pursued, which promise to yield results both of immediate economic importance and of scientific interest.

VI.—*Summary.*

1. The rate of invasion in an apple of a given variety does not remain constant, but may rise or fall in time, according to the variety used and the experimental conditions.

2. A comparison of *Fusarium* strains on various apples shows that the virulence of the strains falls into an order which is independent of the variety of apple and the seasonal changes, but is affected by the temperature of storage. All the saltants examined exhibited a lower virulence than the original strains from which they were derived.

3. The influence of the locality of origin on the resistance of apples of a given variety is shown to be very marked. These differences in resistance can be correlated with differences in chemical composition of the apples.

$$r_{\text{RN.K}} = + 0\cdot737 \quad \left\{ \begin{array}{l} \text{R} = \text{radial advance.} \\[4pt] \text{K} = \text{percentage K in fresh weight of apples.} \\[4pt] \text{N} = \text{percentage N in fresh weight of apples.} \end{array} \right.$$

$$r_{\text{RK.N}} = - 0\cdot282$$

4. Resistance to fungal invasion is complex in nature, and depends on many factors. Some of these are water content, acidity, nitrogen content, potassium content. Low water content, high acidity, high potash content and low nitrogen content are found to be associated with high resistance. The converse is also true.

Rothamsted Experimental Station, for his advice on the statistical side of the investigation.

LITERATURE CITED.

(1) Gregory, F. G., and Horne, A. S., " A Quantitative Study, etc." Part I [The foregoing paper].

(2) Horne, A. S., " Factors affecting the Internal Resistance of Apple Tissues to Fungal Attack," ' Rep. Food Investigation Board, 1924. Department of Scientific and Industrial Research, London.'

(3) Horne, A. S., " Fungal Diseases of Stored Apples," ' Rep. Imperial Botanical Conference, London,' p. 363 (1924).

(4) Brown, W., " Studies of the Genus Fusarium. IV.—On the Occurrence of Saltations." ' Annals of Botany,' vol. 40, p. 223 (1926).

(5) Anon., " Parasitic Fungi of Stored Apples," ' Rep. Food Investigation Board, 1922, p. 39. Depart. of Sci. & Indus. Research, London.'

(6) Anon., ' Rep. Food Investigation Board, 1923. Depart. of Sci. & Indus. Research, London.'

(7) Brown, W., " A Study of Forms of Fusarium occurring on Apple Fruit," ' Rep. Imper. Botan. Conf., London,' p. 339 (1924).

(8) Fisher, R. A., ' Statistical Methods for Research Workers, Edinburgh ' (1925).

(9) Horne, A. S., and Mitter, J., ' Rep. Food Investigation Board, 1925. Depart. of Sci. & Indus. Research, London.' (*In the Press.*)

(10) Archbold, H. K., " Chemical Studies in the Physiology of Apples. II.—The Nitrogen Content of Stored Apples," ' Annals of Botany,' vol. 39, p. 97 (1925).

(11) Brown, Janet W., " Chemical Studies in the Physiology of Apples. V—Methods of Ash Analysis, and the Effect of Environment on the Mineral Constitution of the Apple," ' Annals of Botany,' vol. 40, p. 129 (1926).