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XII—Factors Affecting the Amount of Infection Obtained by Aphis Transmission of the Virus Hy. III

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I—Introduction

Much of the information which can be obtained about a plant virus agent is ultimately derived from the quantity as well as the type of the infections resulting from inoculations to suitable host plants. The number of infections obtained does not depend solely on the nature of the particular virus concerned. It is dependent on other variable factors, such as the efficiency of the means of infection introducing the virus, the susceptibility of the plants receiving it, and the concentration of the virus in the source from which it was obtained. In this paper an attempt has been made to estimate the effect of some of these variables on infection by insects.

The experiments were carried out with the virus Hy. III (Hamilton, 1932) which has the following characteristics listed according to the key characters suggested by Johnson and Hoggan (1925) :

Transmission—Mechanically and by the aphid *Myzus persicae* Sulz. into various Solanaceous plants.
Longevity in vitro—Less than 6 days (crude extract).
Thermal Death point—Below 60° C. („ „).
Potato (*Solanum tuberosum*)—Not susceptible.
Cucumber (*Cucumis sativa*)—Not susceptible.
Tobacco (*Nicotiana tabacum*)—Susceptible.
Symptoms in Tobacco—Intense yellow mottling with tendency to strong dark green vein bands. Considerable stunting and occasionally malformation and necrosis.

In its physical properties and transmission by *Myzus persicae*, Hy. III virus appears to be similar to the cucumber mosaic used for aphid experiments by Hoggan (1933) and by Doolittle and Walker (1928), and possibly to potato virus Y of K. M. Smith (1929 and 1933). In its host range it differs slightly from these.

Hy. III was found to be particularly suitable for the type of experiment which it was proposed to undertake. The symptoms are very characteristic and easy to recognize from the earliest stages, which appear 6 days after inoculation in summer and about 8 or 9 days after in winter. The symptom picture does not vary between summer and winter except that in winter there is rather more stunting and necrosis which, when the inoculated plants are seedlings, frequently causes death. The virus has remained standard in all its properties for six years, during which it has been constantly sub-inoculated through different species of plants and has never been renewed from any outside source.

II—Methods

1—*Insectary and Glasshouses*

The glasshouses were insect-proofed chambers size 10 feet by 10 feet, heated by hot water pipes. The range of temperature is indicated by the following daily averages for 1933–34 : maximum, April to September, 29·17° C., October to

March, 15·73° C.; minimum, April to September, 15·73° C., October to March, 12·94° C.

The insectary was also insect-proofed and was heated by thermostatically controlled electric units, average daily temperature April to September—maximum 21·05° C., minimum 15° C.; October to March—maximum 25·53° C., minimum 15·28° C.; average relative humidity April to September—maximum 76·32%, minimum 59·25%; October to March—maximum 77·27%, minimum 63·32%.

2—*Insects and Culturing Methods*

Radish and turnip plants were used for stock cultures of *Myzus persicae*, with occasional *Hyoscyamus* to increase the vigour of the colonies, but not for immediate use as *Hyoscyamus* is susceptible to the virus. In November, December, and January, if large numbers of insects were to be used, it was found necessary to lengthen the hours of daylight by $2\frac{1}{2}$ hours. Cultures so treated were kept either under a 500-watt electric lamp in a water-cooled glass-topped cage about 18 inches from the light, or else completely uncovered about 3 feet from it. The artificial light was more effective if the plants were uncovered, but this was only possible when the insectary was not being used for any other purpose.

Culturing on to infected and healthy plants was done with small camel-hair brushes. Single aphids and small numbers were placed directly on to the leaf, but, so far as possible, contact between leaf and brush was avoided. When larger numbers were used the insects were dropped into the top of lamp glass cages with which the healthy seedlings had previously been covered, and on these occasions single aphids were treated in the same way as the larger numbers. The tops of the cages were sealed with cellophane covers.

Aphids were infected with the virus by feeding on single leaves detached from the plant, which were placed upright in damp sand, and covered by open ended glass tubes (3 inches × 1 inch). The aphids were inserted into the tubes which were sealed by cellophane covers.

The aphids were generally starved a few hours before feeding on infected leaves by placing 50–100 at a time into cellophane covered petri-dish bases. They were introduced through a small hole in the cellophane cover which was afterwards sealed, and the whole covered by the petri-dish lid to keep it moist. Thus the time occupied by collecting aphids from the stock plants (2–3 hours when large numbers are required) was eliminated from the actual time of culturing on to the infected leaves, and the insects were all in approximately the same condition and equally ready to feed. Single aphids were removed from the plants at the ends of the feeding periods with clean camel-hair brushes; larger numbers were sprayed with a mixture of 0·15% pure nicotine in a 1·00% solution of soft soap.

3—*Plants*

(*a*) *Difference in Susceptibility between Hyoscyamus and Tobacco*—Although the virus Hy. III occurs naturally in *Hyoscyamus*, this plant was found unsuitable for experiments

made at different seasons of the year because the seeds could not be persuaded to germinate between October and March. However, as *Hyoscyamus* was used in many of the earlier weekly experiments, it became necessary to find some means of comparing the results obtained with those of experiments in which tobacco plants were used, in order to find out whether any large error had been introduced by a difference in susceptibility of the two species. The method used was to continue for the following spring and summer with random groups of tobacco and *Hyoscyamus* plants in each experiment. The percentage infections obtained for the two species during this period are given in Table I.

TABLE I—DIFFERENCE IN SUSCEPTIBILITY OF HYOSCYAMUS AND TOBACCO

	Plants used	Plants infected	Percentage infection
Tobacco	1102	573	52·00
Hyoscyamus	838	365	43·56

Tobacco was found to be slightly more susceptible than *Hyoscyamus*, the difference between them in percentage infection being $8{\cdot}44 \pm 2{\cdot}34\%$. The standard error was determined from the weighted means of the 31 repetitions, as the numbers of each plant were not equal in each experiment.

The remainder of the experiments were carried out with tobacco plants only.

(*b*) *Comparison between* Hyoscyamus *and Tobacco as Sources of Infection*—Material for the above experiment was obtained by feeding the aphids for alternate weeks on infected *Hyoscyamus* and tobacco plants, so that it was possible to arrange most of the results according to source of infection as well as for differences in susceptibility. There is no apparent difference in efficiency between the two species as sources of infection (Table II).

TABLE II—DIFFERENCE BETWEEN HYOSCYAMUS AND TOBACCO IN EFFICIENCY AS SOURCES OF INFECTION

	Infection into	Plants used	Plants infected	Percentage infection	Mean
Infection from tobacco . . .	Tob.	500	253	50·6	49·9
	Hyos.	375	177	46·9	
Infection from Hyoscyamus .	Tob.	478	256	53·55	47·82
	Hyos.	361	152	42·10	

(*c*) *Comparison between Tobacco Leaves of Different Ages as Sources of Infection*—The infected plants on which the aphids were fed were all of the same age and inoculation date for each experiment, and were themselves aphid-infected. In practice, infected plants from one experiment were used as sources of infection for the experiment of

the following fortnight, and when the weekly series was broken arrangements were made, whenever possible, to have extra infected plants at the right stage of development.

In selecting leaves for aphid feeding there were two main considerations. In the first place the aphids prefer smooth, hairless leaves, and it is convenient for them to be small. The second consideration was that plenty of virus should be available to the aphids.

For ease of feeding the older leaves were more suitable, as young infected leaves are often malformed and very hairy. For convenience of handling it was essential to use plants which had not developed beyond the stage of the fifth or, at the most, the sixth true leaf.

The relative virus concentration in the different leaves on which the aphids could be fed was estimated from the results of mechanical inoculation. The plants selected as sources of inoculum had been originally inoculated on the first true leaf at the stage when only three leaves were developed, because this was the standard age for seedlings used in all aphid infections.

Three different groups of infected plants of different ages were used for experiments I, II, and III (Table III). Inoculations were made on to healthy tobacco plants using extracts of leaves taken in order of age from these groups of infected plants. Thus the first true leaf provided extract No. 1, the second extract No. 2, and so on to the youngest leaf, the number of extracts depending on the number of leaves which had been developed since the original inoculation.

Counts were made of local starch lesions by Holmes's method (Holmes, 1931), and in this way much smaller numbers of plants were needed than would have been required to obtain the same information by means of aphid infections.

The juice was extracted without addition of water to the leaf pulp and the dilutions were made up from these neat extracts.

The inoculations were made by rubbing with the finger-tip on to half-leaves, about 0·1 c.c. being used for each inoculation. The other half-leaf was rubbed with the same quantity of a control juice composed of equal quantities of each of the extracts used in the experiments. These control halves were used in an attempt to eliminate some of the differences in susceptibility between leaves and plants, because Youden and Beale (1934) found that there is a high correlation between half-leaves. As will be seen below, no advantage was derived from this.

The method of counting and preparing for lesion counts was slightly altered from that of Holmes, in accordance with the special features of the virus used. Hy. III in tobacco gives clearly defined starch lesions varying in appearance from a minute solid spot in the young lesions, through a stage in which 1, 2, or 3 concentric rings are present, which later become rather blurred and diffuse. The best stage for counting is that of the first concentric ring, which occurs on the fourth day after inoculation in summer, and in winter on the fifth or even the sixth day. At 5 p.m. on the fourth day from the date of inoculation the inoculated plants were placed in a cool dark room to remove starch from the leaves, and at 9.0 a.m. on the following day the

inoculated leaves were detached, killed by immersion in boiling water, and decolorized in alcohol. They were then cleared in distilled water for 24 hours and placed for 10 minutes in an aqueous solution of iodine and potassium iodide. After washing, the leaves were floated in water on glass trays and the lesions counted by transmitted light.

Table III shows the results given as lesion counts per half-leaf for each treatment. The last column gives the experimental figures with the number of lesions on the control halves adjusted to a constant value by means of the analysis of covariance (*see* FISHER, 1935, p. 167).

TABLE III—RESULTS OF INOCULATIONS FROM LEAVES OF DIFFERENT AGES

Experiment No.	Number of leaf used as source of inoculum	Mean number of lesions per half-leaf. Control	Experimental Actual	Experimental Adjusted
I	1	47·45	5·18	4·19
(25.7.35)	2	32·27	2·36	2·55
	3	27·90	*4·81*	*5·34*
	4	31·09	3·63	3·91
S.E. mean of 11 observations			± 0·952	± 0·754
II	1	12·00	6·67	7·07
(30.10.35)	2	9·00	0·33	1·60
	3	16·33	13·67	12·82
	4	11·33	*35·00*	*35·60*
	5	18·33	17·00	15·57
S.E. mean of 3 observations			± 3·10	± 3·111
III	1	63·56	4·00	3·25
(9.8.35)	2	65·56	0·56	− 0·30
	3	30·66	4·56	5·64
	4	49·16	*9·00*	*9·05*
	5	50·00	4·16	4·17
	6	41·83	2·00	2·52
S.E. mean of 6 observations			± 1·514	± 1·404

	Control	Experimental
Dilution for experiments I and III	1/100	1/1000
Dilution for experiment II	1/100	1/100

The results for experiments II and III show a concentration of virus for the fourth leaf which is significantly greater than those for the other leaves used. The difference between leaves for experiment I is not quite significant, but the adjusted figures agree with the results of the other two experiments, except that in the younger plants the third leaf gives the highest concentration. In no experiment was the error greatly decreased by the use of the regression on the controls, which

indicates that the increased accuracy obtained was not sufficiently great to justify the use of half the experimental material for control purposes. This point will be discussed in the next section.

These results indicate that the first two leaves of a young plant, though the most satisfactory for aphid feeding, are not the most efficient sources of infection. The third leaf was therefore chosen on all possible occasions, but where the supply of third leaves was not sufficient, a random selection of first and third leaves was used, as the fourth was generally deformed and very hairy, and the second appeared to have a low virus concentration.

(*d*) *Differences in Susceptibility between Tobacco Leaves of Different Ages*—Experiment II above was designed so that it might also yield some information about susceptibility of the various leaves to inoculation. Such information applies only to mechanical inoculation, and susceptibility to aphid inoculation will not necessarily be the same in all respects, but some factors at least are probably common to both methods of infection.

A preliminary grouping to find the principal source of variation *within plants* was made by dividing the leaves into blocks according to age. This arrangement gave the following total numbers of lesions for all treatments :—

3rd leaf		4th leaf		Total	
Experimental	Control	Experimental	Control	Experimental	Control
98	1083	49	741	147	1824

The analysis of variance for the experimental figures :—

	D.F.	Mean Square
Blocks (3rd leaf *v.* 4th leaf)	1	66·69
Blocks × Treatments	5	8·56
Treatments	5	48·65
Error	24	13·75

shows that the mean square variance for blocks is significant, and this is true also for the controls and for the adjusted figures.

In order to obtain more exact information on this point and also, though this was not of immediate interest from the point of view of aphid infection, to examine the correlation between half-leaves, a uniformity trial was made using the same virus extract for all treatments. In this experiment 10 similar plants were selected and independent halves of the second, third, and fourth leaves rubbed at random with a dilution of 1/100 infected plant extract. The randomization was done by cutting from a pack of 60 numbered cards, one for each of the 60 half-leaves. The results are given in Table IV.

The upper part of Table IV gives the results for each inoculation on the third and fourth leaves. No figures were obtained for the second leaves as they took a very severe infection which resulted in large areas of necrosis so that accurate counts were impossible. The lower part of the table gives the analysis of variance on a

plant basis for the totals of 4 figures. Comparison of the mean squares resulting from this analysis brings out the following points :

1. The third leaf has given a significantly higher mean number of lesions than the fourth, the difference being 67·4 with a standard error of $\pm$ 22·47 ($= \sqrt{504 \cdot 75}$). In view of this it appears that advantage would be gained in an experiment by

TABLE IV—RESULTS FOR LESION COUNTS ON THIRD AND FOURTH LEAVES

Plant number	Third leaf		Fourth leaf		Difference (Third leaf *v.* Fourth leaf)
	Right half	Left half	Right half	Left half	
1	56	73	32	50	47
2	33	32	16	35	14
3	47	58	22	48	35
4	58	48	47	50	9
5	72	68	16	21	103
6	75	56	66	83	−18
7	40	60	29	49	22
8	76	101	17	39	121
9	179	106	23	18	244
10	77	63	18	25	97
Total	713	665	286	418	674

Analysis of variance (on a plant basis—total of 4 figures).

	Degrees of freedom	Sum of squares	Mean square
Between plants	9	31,805·6	3,534·0
Within plants between leaves.			
Third leaf *v.* fourth leaf	1	53,286·4	53,286·4
Remainder	9	45,427·6	5,047·5
Within leaves between half-leaves.			
Right half *v.* left half	1	705·6	705·6
Remainder	19	19,454·4	1,023·9
Total	39	150,679·6	4,023·6

keeping these leaves in separate blocks so that the average differences between them might be eliminated from the treatment comparisons.

2. This is supported by the fact (obtained from comparison of the mean square variance *between plants* with the remainder) that the variation *between plants* is no greater than *within plants between leaves*, so that nothing would be gained by restricting blocks to single plants.

3. There is apparently no difference between right and left halves of leaves as is shown by a comparison of the two mean squares for *within leaves between half-leaves*.

The variation *between leaves* is, however, significantly greater than that *within leaves* so that there is a correlation between the numbers of lesions on halves of the same leaf; but it has already been shown that this correlation is not sufficiently great to increase the accuracy of experiments where corresponding half-leaves are used as controls. Much greater advantage would probably have been gained by using each half-leaf independently for experimental inoculations, thus doubling the number of observations. Similarly, SAMUEL, BEST, and BALD (1929) found, for spotted wilt disease of tomatoes, that no advantage was gained by the use of control half-leaves in this type of experiment. This may be due to the fact that with rubbing experiments, it is inexpedient to follow each experimental inoculation with its control, because of the danger of accidental infection. Consequently the experimental and control inoculations were done in separate groups, which tends to decrease the variation between leaves within plants, and between plants, at the expense of that between half-leaves.

As the results showed a considerable difference in susceptibility between leaves of different ages, it was necessary to use the same leaf consistently for aphid infections whenever possible, or else to use efficient randomization between different leaves. The older leaves appeared to be the most susceptible and as they were also the most suitable morphologically, aphids were fed on the first true leaf or a random selection of first and second. This does not apply to occasions on which large numbers of aphids were fed, or to those in which the aphids were dropped into the glass cages. In these experiments the aphids selected their own feeding places.

III—APHID INFECTIONS

1—*Seasonal Variation*

A possible source of error when experiments are done at different times of the year is seasonal variation, and in glasshouse conditions there appears to be a considerable seasonal effect on the percentage infection produced by *Myzus persicae* with the virus Hy. III.

Fig. 1 shows the weekly percentage infection obtained out of 30 plants per week for 14 months, 1933–34. The results for 5 and 10 aphids were used together. The total hours of sunshine for the same weeks are also shown.

A winter maximum of percentage infection is reached which continues from the end of October to the middle of January and corresponds to the period of least sunlight. The positions of the points suggest a negative correlation between deviations from the smooth general trend of the two curves, indicating that the hours of sunshine for the week in which the infection took place, or possibly in the previous week, have a direct effect upon the number of infections obtained. This, however, was not significant. There appeared to be a negative correlation with average mean daily temperature for the current week, though this was not quite significant. (Variance due to regression 395·4; residual variance 189·84.) This effect

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was probably due to the lengthening of the penetration time (*see* p. 475) by a fall in relative percentage humidity, resulting from high temperature, and in any case only accounts for a very small fraction of the variance.

Fig. 2 gives the two curves representing the mean weekly percentage infection for 5 aphids per plant over the period October to March for the years 1933–34, and 1934–35. The gradual rise to a maximum for December and January and a drop towards the following spring is clearly shown in both curves, so that the effect appears to be a general one and not peculiar to 1933–34. The 1934–35 curve, particularly the autumn part of it, is smoother than that of the previous year. No corresponding

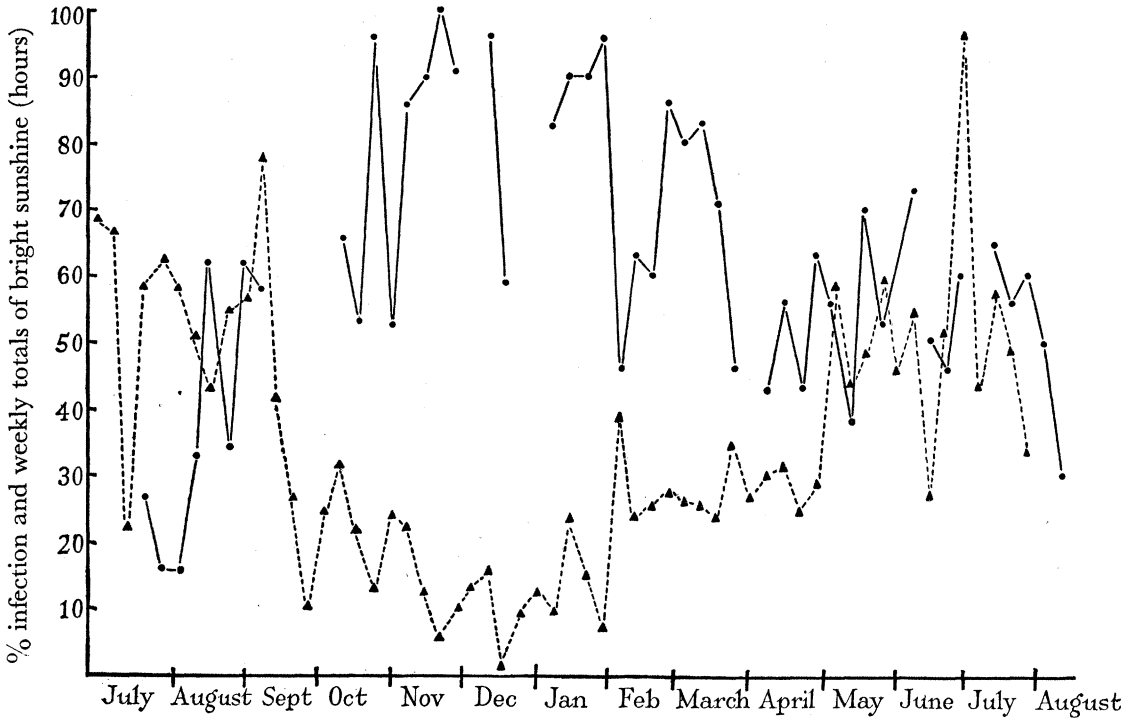

FIG. 1—Seasonal variation in percentage infection, with weekly sunshine curve. • % infection ; ▲ weekly sunshine curve.

differences were recorded for meteorological conditions, and this result is probably attributable to improved technique and greater experience in the measures necessary for controlling conditions in the insectary.

The two curves do not correspond particularly well in their weekly variations except at one point, namely, the large fall in percentage infection between the second and third week in December. This follows closely on periods, terminating on 27 November, 1933 and 4 December, 1934, when degeneration of the insect cultures from lack of light, or indirectly from starvation due to etiolation of the food plants, necessitated a break in the experimental series and artificial light had to be used to restore them. This upset the experimental routine so that at first rather old and then very young plants had to be used as sources of infection, which possibly accounts

for the observed fall. The use of artificial light on the aphid cultures did not otherwise appear to affect the general trend of percentage infection.

These results show a clear seasonal variation in percentage infection, and this variation occurs for all numbers in aphids used per plant. The following figures give the annual range in percentage infection for each aphid number. They are taken from the monthly averages as the weekly totals show a very wide variation. The range is greater for the smaller aphid numbers than for the larger ; this type of

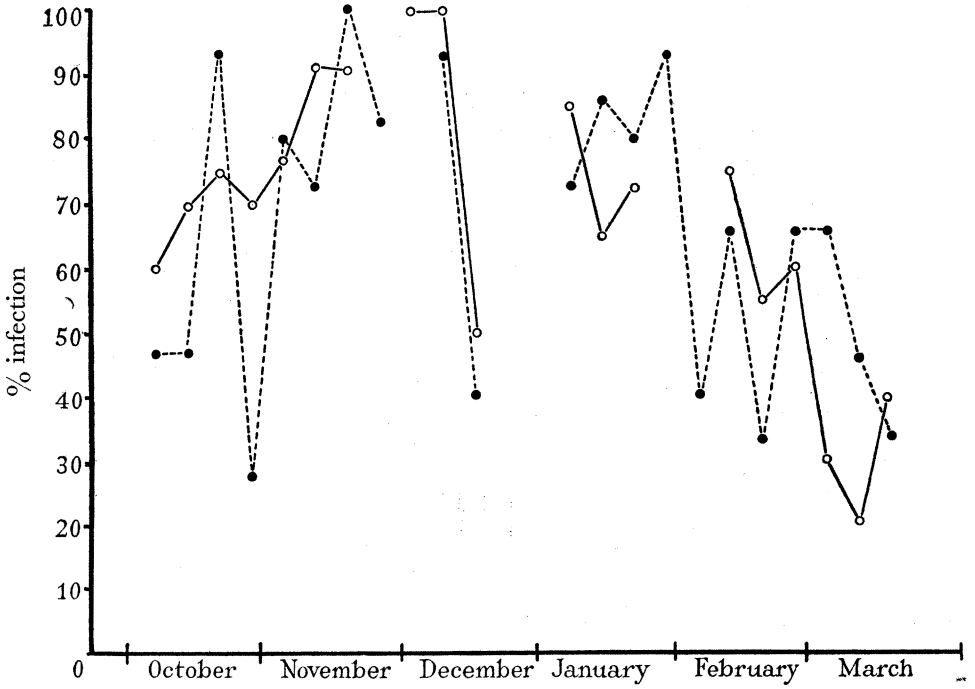

FIG. 2—Winter and Spring variation in percentage infection for two consecutive years, 5 aphids per plant. • 1933–34 ; o 1934–35.

variation is also found in other experiments when different aphid numbers are used (*see* page 471).

For	1 aphid	between	5	and	40%.
,,	5 aphids	,,	20	,,	80%.
,,	10 ,,	,,	40	,,	95%.
,,	20 ,,	,,	75	,,	100%.

The absence of significant correlation between individual weekly totals and meteorological conditions is probably due to the interaction of many other factors which tend to mask or nullify these effects ; for example :—

1. Biological errors due to variation in the condition of the aphid cultures and in technique.

2. The inadequacy of hours of sunshine as a criterion of light effect. Total radiation in the insectary and glasshouses would probably have been better, but this was not recorded.

3. The possibility that susceptibility to infection is increased as rate of plant growth increases. Increase in hours of daylight from the beginning of March should, according to the general trend of the curve, cause a decrease in percentage infection but coincides with the onset of rapid spring growth in the plants, and the general descent of the curve would be checked until the flush of spring growth ceases at the end of June. This would account for the two periods of minimum percentage infection shown by the curves in March–April, and later in July–August.

2—*Effect of Number of Aphids Used per Plant*

Hoggan (1933), using cucumber mosaic, has shown that the amount of infection obtained with *Myzus persicae* as a vector varies with the number of aphids used. With Hy. III the results from 240 infections in tobacco for 1, 5, 10, and 20 aphids are given in the top row of the upper part of Table V, and it can be seen that the same is true for this virus.

Table V—Distribution of Infection on 960 Plants

No. of aphids per plant	1	5	10	20
No. of plants (out of 240) infected	28	127	163	190
Expected number, susceptibility constant	24	99	157	211
Expected number, susceptibility varying	25	100	150	199

The figures were obtained from the results of 16 weekly experiments carried out between April and September, 1934. The 60 plants used in each experiment were divided into batches of 15 which were treated alike in respect of aphid number but were also subjected to different feeding times in batches of 3 plants.

If the probability of infection, p, by a single aphid were constant and independent of whether other aphids were feeding on the plant, so that the probability of non-infection, q, by this aphid is $q = 1 - p$, then the probability of non-infection by x aphids all feeding simultaneously would be q^x, and consequently the probability of infection would be $1 - q^x$, since only when none of the x aphids produces infection will the plant as a whole escape infection.

The second line of Table V shows the expected number of infected plants with $p = 0{\cdot}1005 \pm 0{\cdot}0047$, which is the value given by the maximum likelihood solution. The agreement is not perfect, there being too few infected plants in the 20 class and too many in the others, particularly the 5 class.

If the probability of infection varies from batch to batch, similar figures may be deduced, estimating a different value of p for each batch of 60 plants. The last line of Table V shows the expected values obtained on this hypothesis. The observed and expected numbers of infected plants and the corresponding values of p for each batch are shown in Table VI. p ranges from $0{\cdot}05$ to $0{\cdot}15$. The total observed and expected values now agree somewhat better, but there is still evidence of a tendency for the number of infected plants to be lower than expectation for 20 aphids and above expectation for 1, 5, and 10 aphids.

TABLE VI—DISTRIBUTION OF INFECTION FOR WEEKLY RESULTS OF 16 EXPERIMENTS, FIRST SERIES

Experiment No.	1 Aphid		5 Aphids		10 Aphids		20 Aphids		q
	A.V.*	M.L.V.	A.V.	M.L.V.	A.V.	M.L.V.	A.V.	M.L.V.	
1	0	*1·0*	7	*4·6*	7	*7·7*	11	*11·5*	0·93
2	0	*1·5*	7	*6·1*	12	*9·8*	12	*13·2*	0·90
3	0	*1·2*	6	*5·1*	7	*8·5*	11	*12·2*	0·92
4	2	*1·8*	9	*7·1*	10	*10·8*	13	*13·8*	0·88
5	2	*1·8*	10	*7·1*	7	*10·8*	15	*13·8*	0·88
6	2	*1·2*	12	*5·1*	12	*8·5*	5	*12·2*	0·92
7	3	*1·5*	8	*6·1*	13	*9·8*	10	*13·2*	0·90
8	1	*2·2*	10	*8·3*	12	*12·0*	14	*14·4*	0·85
9	2	*1·4*	5	*5·6*	9	*9·2*	13	*12·7*	0·95
10	3	*1·0*	7	*4·6*	6	*7·7*	11	*11·5*	0·93
11	3	*1·5*	6	*6·1*	12	*9·8*	11	*13·2*	0·90
12	3	*2·1*	10	*8·0*	12	*11·7*	13	*14·3*	0·86
13	4	*1·6*	7	*6·6*	11	*10·3*	12	*13·5*	0·89
14	0	*1·6*	6	*6·6*	11	*10·3*	14	*13·5*	0·89
15	2	*1·8*	6	*7·1*	15	*10·8*	12	*13·8*	0·88
16	1	*1·5*	11	*6·1*	7	*9·8*	13	*13·2*	0·90
Total	28	24·7	127	100·3	163	149·8	190	198·6	

* A.V. = Actual Value; M.L.V. = maximum likelihood value; q = probability of non-infection by single aphid.

Discrepancies of this nature would arise if the probability of infection varied from plant to plant, as is easily seen if we consider the limiting case in which some plants become infected on exposure to any infection and others are totally immune. If all aphids carried infection, the numbers of plants infected would be equal for all the numbers of aphids per plant.

Such discrepancies might also arise if different aphids carried different amounts of the infective material, as might happen if different leaves of the host plant were infected differently. In the absence of proper randomization, the whole of a batch of 20 aphids might then tend to be highly infected, or only slightly infected, and this would give rise to the type of discrepancy observed. Therefore, a second series of experiments was carried out in the spring of 1935, using larger numbers of plants over a period of 10 weeks so that the material used was more homogeneous, and the experiments less widely separated in time, than in the previous ones.

The procedure of these experiments was as follows:

On each of forty plants one aphid was placed and immediately 10 plants were withdrawn at random, forming the group receiving one aphid only. The remaining 30 plants received four more aphids given singly to each plant in its turn. Ten were withdrawn at random and constituted the 5-aphid group. The 10- and 20-aphid groups were formed in the same way. Thus, over the whole series, 100 plants were

used for each aphid number. The results of the experiments with the expected values calculated from the maximum likelihood value of q for the total figures ($q = 0{\cdot}828$) are given in Table VII.

TABLE VII—DISTRIBUTION OF INFECTION FOR WEEKLY RESULTS OF 10 EXPERIMENTS, SECOND SERIES

Experiment No.	1 Aphid	5 Aphids	10 Aphids	20 Aphids
1	4	9	10	10
2	2	6	10	9
3	2	2	9	9
4	0	5	7	10
5	3	7	8	9
6	2	9	10	10
7	2	6	10	10
8	1	4	10	9
9	2	6	8	9
10	0	6	9	9
Number of plants infected out of 100	18	60	81	94
Expected number, susceptibility constant . .	17·2	61·1	84·4	97·7

The expected values now fit very closely to the figures obtained so that part of the discrepancy found in the previous series of results has been eliminated by the later modifications in technique and materials.

If the infections were not local and independent but could be caused by the cumulative effect of individually inadequate doses, discrepancies of the opposite type would occur, the spread of the observed values being wider than that of the expected values. An effect of this kind may conceivably be masked by either or both of the causes of disturbance discussed above, but, in the absence of other evidence, each aphid infection may reasonably be assumed to be essentially local and independent.

3—*Effect of Varying Feeding Times on the Healthy Plants*

In these experiments aphids were fed for 12 hours on infected leaves, then placed in batches of varying number on to groups of healthy seedlings, and allowed to feed for varying periods. It was desired to use as large a number of plants as possible for each treatment and this necessitated numerous repetitions at weekly intervals. In order to equalize so far as possible the effect of season and other special conditions over the various repetitions, they were continued, with a few breaks, throughout the year 1933–34. The weekly mean percentage infection gave the data on seasonal variation in percentage infection which was discussed in § III.

The first experiments were carried out with groups of 5 and 10 aphids, between July and September, 1933. For each aphid number 15 plants were used and sets of 3 from each group were sprayed after periods of 3, 6, 12, 24, and 48 hours' feeding.

The 20-aphid groups were carried on in the same way as the previous ones from September until the end of November, when they were discontinued owing to shortage of aphids. The winter percentage infection curve reached its highest values at this time, and the 20-aphid groups were giving a regular weekly result of 100% infection for all times so that the interruption probably caused little loss of information. The 20-aphid treatment was restarted in February, 1934, when the percentage infection was falling again.

Experiments with single aphids had not been started in July with the 5- and 10-aphid groups because previous experiments done in spring and summer had shown

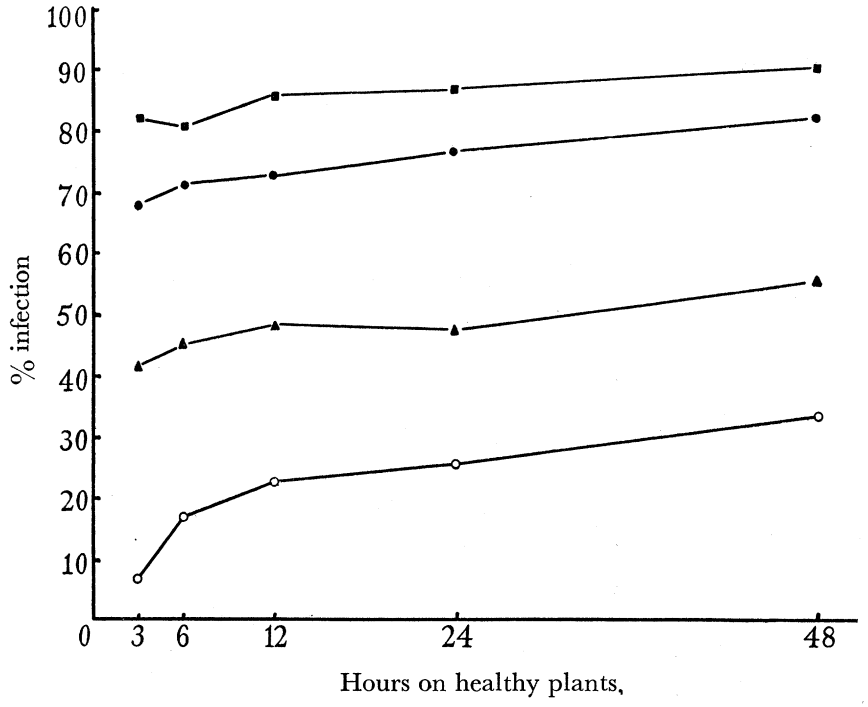

FIG. 3—Effect of increasing feeding periods on the healthy plant for different numbers of aphids. ■ 20 aphids ; • 10 aphids ; ▲ 5 aphids ; ○ 1 aphid.

very small or negative results. When the higher winter percentage infection became apparent in December, 1933, single aphid groups were started and continued until the end of the experiment. They gave relatively high percentage infections in the winter and early spring, the figures for the following summer being small but consistent with the other results.

The results of these experiments are given in Table VIII, and fig. 3 shows the percentage infection plotted against time on healthy plant for each aphid number.

The linear regressions, which measure the average increase in percentage infection per hour, are also given in Table VIII, and they are highly significant.

The increase in percentage infection per hour tends to lessen as the numbers of aphids increase. This is not surprising since for the higher aphid groups many of

the winter results show 100% or nearly 100% infection for all times on the healthy plant.

There does not appear to be any distinct interval of time before infection is possible which could be called an "incubation period", such as is described by KUNKEL

TABLE VIII—EFECT ON PERCENTAGE INFECTION OF TIMES OF FEEDING ON HEALTHY PLANTS

Hours on Healthy Plant	1 Aphid		5 Aphids		10 Aphids		20 Aphids	
	Plants used	% infection	Plants used	% infection	Plants used	% infection	Plants used	% infection
3	114	6·6	144	41·6	141	68·1	72	81·8
6	114	16·5	144	45·1	138	71·1	72	79·7
12	114	22·6	141	48·2	135	72·7	72	85·5
24	114	25·5	138	47·1	135	76·5	69	86·3
48	114	27·8	138	55·7	135	82·5	69	89·3
Increase in percentage infection per hour*	0·37 ± 0·161		0·27 ± 0·0519		0·30 ± 0·0294		0·18 ± 0·055	

* Calculated from the linear regression coefficient.

(1926), STOREY (1928), and others. Their viruses, however, appear to have a different type of relation to their vectors (*see* p. 484). The suggestion of an incubation period in the insect for Hy. III made in my previous paper HAMILTON (1932) was due to small numbers of plants having been used at a season of low percentage infection, so that only their periods of maximum infection at 24 and 48 hours gave positive results.

HOGGAN (1933), using cucumber mosaic, did not find any difference in percentage infection for feeding periods of from $\frac{1}{4}$ hour to 24 hours on the healthy plant. This may be due to differences in the nature of cucumber mosaic and Hy. III, but it is also possibly accounted for by the fact that large numbers of aphids, 10 or 20 per plant, were used so that the increase per hour would be relatively small.

The shape of the curves is of interest in that the upper pair of lines are straight and probably do not pass through zero ; the regions corresponding to the shortest times in the lower pair are curved and might conceivably do so.

In order to find out the effect of shorter feeding periods another series of experiments was carried out between September, 1934, and January, 1935. The treatments consisted in culturing groups of 1 and 5 aphids on to batches of 5 plants each and allowing them to feed for periods of $\frac{1}{4}$, $\frac{1}{2}$ (for single aphids only), 1, 3, 6, and 12 hours. Some short term infections had already been done for 5 aphids in the summer of 1934 and these are included in the 5-aphid results. The results are given in Table IX and rise in percentage infection is plotted against time in fig. 4.

For the whole period of 24 hours the slopes are somewhat steeper than was found for the 48-hour periods, the rise for the 1-aphid line being about 1% per hour and

for the 5-aphid line being 0·8% per hour against 0·37% per hour for the 1-aphid 48-hour curve, and 0·27% for the 5-aphid curve. This indicates that the rise is faster for the shorter feeding periods, and that the suggestion of departure from linearity in the region corresponding to these times is probably justified. There is no suggestion from the curves that infection is not instantaneous, for if there were

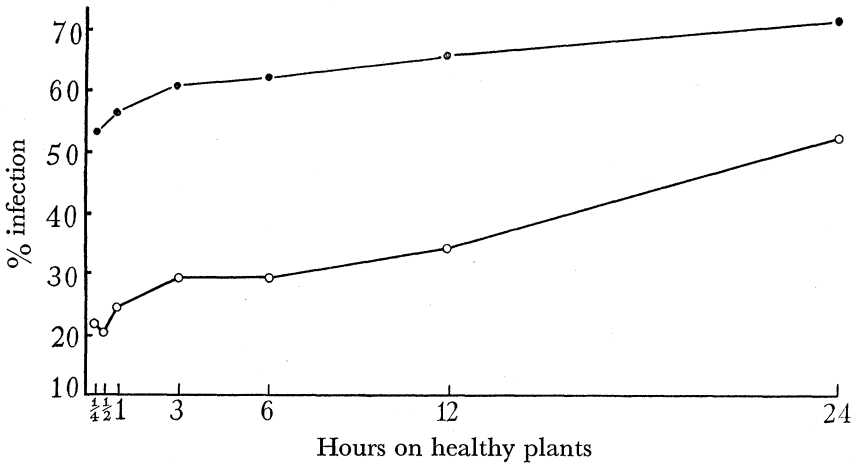

FIG. 4—Effect of shorter feeding periods on the healthy plant. • 5 aphids ; ○ 1 aphid.

TABLE IX—EFFECT ON PERCENTAGE INFECTION OF SHORT PERIOD FEEDING ON HEALTHY PLANTS

Time on Healthy plant	1 Aphid		5 Aphids	
	Plants used	% infection	Plants used	% infection
¼-hour	72	22·2	114	53·5
½-hour	72	20·2	—	—
1 hour	72	26·4	114	56·1
3 hours	72	27·8	111	60·5
6 hours	69	27·5	111	61·3
12 hours	63	34·5	104	69·0
24 hours	69	47·6	104	74·0
Increase in percentage infection per hour*	1·01 ± 0·107		0·82 ± 0·142	

* Calculated from the linear regression coefficient.

any appreciable lag, zero percentage infection would be shown for a positive value of the time.

The actual percentage figures are higher in fig. 4 than in fig. 3, because most of the infections were done in the winter period, whereas the previous results were totals for a whole year, but the general trend for all curves is the same. It is remarkable

that the increase shows no signs of falling off at the 24- and 48-hour periods ; this may be due to insufficient data or to increased susceptibility of the plants due to aphid injury (*see* p. 486).

4—*Preliminary Experiments on the Effect of Feeding Time on Infected Plants, Short Term Feeding and Consecutive Infection*

A further series of infections with single aphids was carried out between January and early May, 1935, to find out the effect of very short feeding periods on healthy plants, and of variation in feeding time on infected plants. Weekly batches of 10 plants for each treatment were used. (The arrangement is shown in Table X.) Each experiment was divided into two halves, half the aphids being fed on the infected leaves for 12 hours (9.30 p.m. to 9.30 a.m.) and half for 3 or 5 minutes. The aphids were immediately cultured on to batches of 10 healthy seedlings which were called the " A " plants. After their allotted periods of 3 or 5 minutes they were transferred directly to a second series of 10 seedlings, called the " B " plants, in order to find out whether they were capable of infecting more than one plant without further access to sources of infection. Consecutive infections are those in which an aphid has infected a second healthy plant after feeding on the first. The aphids were allowed to feed on the " B " plants for 2 hours.

To obtain the 3- and 5-minute feeding periods on both healthy and infected plants, individual aphids were watched through a lens until they had settled into the feeding position ; that is to say, with the abdomen horizontal and close to the leaf, the antennae laid back in line with the body, and the rostrum at right angles to the leaf and touching it. They were only considered as fed when they had held this position continuously for the requisite time.

The time occupied by the aphid in finding a suitable feeding place and settling down to feed, is referred to as the " Penetration Time ". For 95% of the aphids, the penetration time was less than 10 minutes, 3% took between 10 and 15 minutes, and about 2% longer than 15 minutes ; but some of these may have been damaged and would not have fed at all. The average penetration time for 560 aphids was 4·88 minutes.

It was noted that penetration time was longer on some days than on others and this variation was found to be negatively correlated with relative humidity in the insectary at the time of feeding. Fig. 5 shows the relation between penetration time and relative humidity at time of feeding. The time chosen for ease of reading the hydrograph record was 10 a.m., but the actual series of cultures took about $1\frac{1}{2}$ hours to complete. The relative percentage humidity was recorded by a recording hair hygrometer.

The linear regression coefficient of penetration time on relative humidity, $-0{\cdot}129 \pm 0{\cdot}031$, was highly significant and accounted for 52·5% of the variance.

The preliminary results for short feeding periods are given in Table X in which the number of infections obtained from feeding on the first plant of each pair are given in the columns headed A. The columns headed B give the total number of infections

in the second series of plants, and the C columns show the number of consecutive infections in which both A and B were infected. The fact that B infections occur when A is not infected may have been due to failure on the part of the observer to ensure proper feeding on A, but later experiments indicate that this is unlikely, so that a large proportion of these also represent consecutive infections (p. 483).

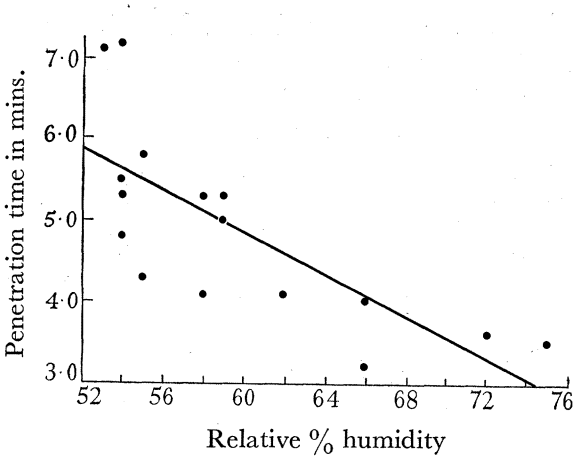

FIG. 5—Effect of relative humidity on " penetration time ".

TABLE X—PERCENTAGE OF INFECTIONS AND CONSECUTIVE INFECTIONS OBTAINED BY SINGLE APHID FEEDING FOR VARIED TIMES ON INFECTED AND HEALTHY PLANTS

	Feeding time on healthy plant A					
	3 minutes			5 minutes		
Feeding time on infected plant	A	B	C	A	B	C
3 minutes	74	40	30			
5 minutes				64	40	31
12 hours	18	15	10	28	15	13

Feeding time on the B plant was 2 hours throughout

This preliminary experiment gave a somewhat unexpected result, in that the percentage infection decreased with increasing time on the infected plant, and a very much higher number of infections was obtained for 3 and 5 minutes' feeding than for 12 hours. There is agreement with previous results in the increase in percentage infection with increasing time on the healthy plants.

The aphids show themselves capable of infecting more than one plant consecutively without intermediate access to any source of infection. This is not remarkable in itself, for many insect vectors of virus diseases retain their infectivity for long periods, but it was not in agreement with the results of previous experiments in which two hours' feeding period was allowed on the first healthy plant and in which no consecutive infections were obtained.

Such anomalies might be caused by variation in the probability of infection due to other factors, such as have been described in previous sections of this paper, and it was thought that some advantage would be gained by more comprehensive investigations.

5—*Factorial Experiment*

A second series of experiments was arranged on a factorial design (Fisher, 1935). In materials and technique they were similar to the experiments described in § III, 4, except that 3 plants instead of 10 were used for each treatment and consecutive treatment. Six variants of feeding time on infected and healthy plants were tested giving 36 combinations of treatments, and the whole was repeated for consecutive infections, giving 72 combinations. Thus the total number of plants used in each experiment was 216, and the experiment was repeated for 10 weeks, giving 2160 plants in all. The general arrangement is shown in Table XI in which columns A, B, and C are the same as in Table X.

The six variants of each factor, that is to say the feeding time on infected plants and on the first healthy plants, or " A " plants, were 2 minutes, 5 minutes, $\frac{1}{4}$, 1, 6, and 12 hours. For all the two-minute and five-minute feedings the aphids were watched and the feeding period timed from the moment of penetration. For the quarter-hour feedings the average " penetration time " was allowed in addition to the treatment feeding time, but for the longer periods the penetration time was neglected. On the second series of healthy seedlings, namely, the " B " plants, all the aphids were allowed to feed for twenty-four hours.

Such a long series of cultures and sub-cultures could not be carried out even approximately at the same times of day. The programme of work was, however, planned so as to equalize, so far as possible over the different treatments, the times of day at which these treatments were started. This was most difficult for some of the six- and twelve-hour feedings, which could only be varied by starting some in the morning and feeding till night and others at night and allowing them to feed till morning.

The actual conditions in which the aphids were fed were not very variable, for the humidity within the lamp glass cages varied very little throughout the day, and all the watched feedings were done under small bell jars in which the air was kept at a high relative humidity by means of an atomizer. After dark the feedings were carried on by artificial light. No marked variation in penetration times or in the general behaviour of the aphids was observed for any particular time of the day. The main danger was the possibility of variation in susceptibility of the plants during the course of the day, but any effect on the treatment comparisons was presumably eliminated by altering the times at which treatments were started from one extreme to the other.

The results for the 10 experiments are given in Table XI which shows the number of infections for each treatment, and the totals for all variants of each factor.

The results confirm those of the preliminary experiment concerning short term feeding on the infected plant, and support those already given (p. 473) for increase in

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TABLE XI—RESULTS OF FACTORIAL EXPERIMENT

Time on infected plant.	Time on first healthy plant: 2 min.			5 min.			15 min.			1 hour			6 hours			12 hours			Total		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
2 mins.	17	11	11	20	8	8	13	4	2	18	0	0	16	1	1	23	0	0	107	24	22
5 mins.	11	7	2	13	10	8	10	0	0	14	0	0	18	0	0	15	0	0	81	17	10
15 mins.	10	4	0	6	3	3	10	2	2	9	0	0	9	0	0	18	0	0	62	9	5
1 hour	3	3	2	3	2	1	2	0	0	2	0	0	7	0	0	4	0	0	21	5	3
6 hours	3	3	1	1	0	0	7	2	0	6	2	2	6	0	0	12	0	0	35	7	3
12 hours	6	4	3	9	4	1	8	3	0	2	0	0	4	0	0	9	0	0	38	11	4
Total	50	32	19	52	27	21	50	11	4	51	2	2	60	1	1	81	0	0	344	73	47

A = Total number of infections on first healthy plant.
B = Total number of infections on second healthy plant.
C = Total occasions on which A and B were both infected by the same aphid.

infection with time allowed on the healthy plant. Those for consecutive infections show that there is a decrease in consecutive infection with increasing time on the first healthy plant, and this is also in agreement with expectation.

The data from which figs. 6 and 7 are plotted are given as percentages with their means and standard errors in Table XII.

TABLE XII—SUBSIDIARY TABLE SHOWING PERCENTAGE INFECTIONS FOR TIMES ON INFECTED AND HEALTHY PLANTS

Time on infected plant		Time on healthy plant: 2 m.	5 m.	15 m.	1 hr.	6 hr.	12 hr.	Mean	
Part I	2 m.	56·66	66·66	43·33	60·00	53·33	76·66	59·44	Standard error of Mean ± 3·57
	5 m.	36·66	43·33	33·33	46·66	60·00	50·00	45·00	
	15 m.	33·33	20·00	33·00	30·00	30·00	60·00	34·44	
	Mean	42·22	43·33	36·66	45·55	47·77	62·22	S.E. of Mean ± 5·03	
Part II	1 hr.	10·00	10·00	6·66	6·66	23·33	10·33	11·66	Standard error of Mean ± 2·70
	6 hr.	10·00	3·33	23·33	20·00	20·00	40·00	19·44	
	12 hr.	20·00	30·00	26·66	6·66	10·33	30·00	21·11	
	Mean	13·33	16·66	18·88	11·11	18·88	26·66	S.E. of Mean ± 3·50	
Mean of Parts I and II		27·77	28·88	27·77	28·33	33·33	45·00	31·85	S.E. ± 3·06

To gain increased accuracy in the estimation of error the data are divided into two parts, as a change in the effect of time on infected plant appears at about 1 hour. The large differences in numbers of infected plants for periods less than 1 hour and greater than 1 hour, cause these two parts of the analysis to have different standard errors. Part I is for 2, 5, and 15 minutes on the infected plant, and all times on the healthy plant, and Part II is for 1, 6, and 12 hours on the infected plant, and all times on the healthy plant. The analysis of variance is given in Table XIII.

TABLE XIII—ANALYSIS OF VARIANCE

NUMBER OF PLANTS INFECTED OUT OF THREE FOR TIMES OF APHID FEEDING ON INFECTED AND HEALTHY PLANTS

	Degrees of Freedom	Mean Square: Part I	Part II
Time on infected plants	2	8·505	1·373
Time on healthy plants	5	2·055	0·956
Interaction	10	0·806	0·818
Occasions	9	2·543	0·422
Error	153	0·684	0·401
Total	179		

The fraction representing the variance due to treatments is divided into time on infected plant, time on healthy plant, and their interaction, and it is convenient to examine the results separately in this order.

(*a*) *Time on Infected Plants*—Fig. 6 shows the variation in percentage infection for different times on infected plants, each point giving the results of 60 observations. In this graph, and in figs. 7, 8, and 9, the percentages are plotted against the logarithms of the numbers of minutes instead of the actual times, as this enables the positions of the points to be more evenly spaced.

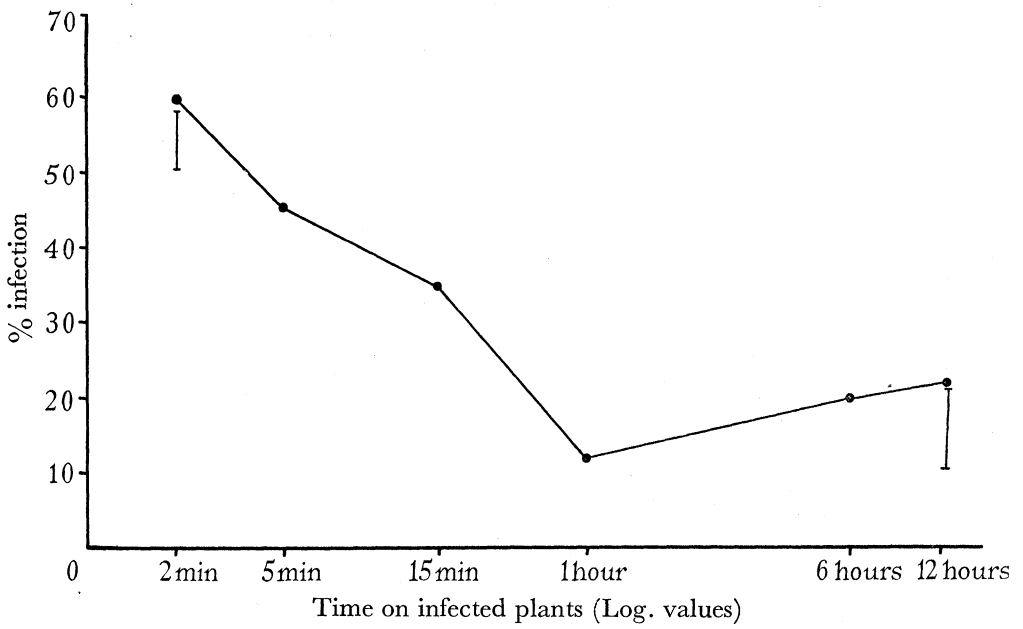

FIG. 6—Effect of varying times on the infected plant, for all times on the healthy plant. Vertical lines = significant differences (S.E. × 3).

The high percentage infection from 2 and 5 minute feedings indicated in the preliminary experiment is here clearly demonstrated. From its highest point of 60% after only 2 minutes' feeding the percentage infection drops rapidly to a value of 11% for 1 hour's feeding, and then rises again very slowly to 21% for 12 hours' feeding. The form of the curve suggests that the rise may continue after 12 hours. The differences between all times from 2 minutes to 1 hour are significant, and also the rise from 1 hour to 12 hours. This can be seen from the analysis of variance, from the standard errors given in Table XII, and from the vertical lines representing significant differences given on fig. 6.

(*b*) *Time on Healthy Plants*—The two unbroken lines on fig. 7 represent the percentage infection for times on the healthy plant. The points on the upper curve represent the means of the values for 2, 5, and 15 minutes on the infected plant, those on the lower one being the means of the values for 1, 6, and 12 hours on the infected plant. Each point therefore represents the results of 30 observations.

The broken line represents the mean percentage infection for all times on infected plant. The significant differences are given as vertical lines beside each curve. Both curves have a significant regression of percentage infection on time as is shown by the analysis given in Table XIV :—

TABLE XIV

		Degrees of freedom	Variance Part I	Variance Part II
Time on healthy plant	Regression	1	5·762	2·333
	Remainder	4	1·087	0·611
	Error	153	0·684	0·401

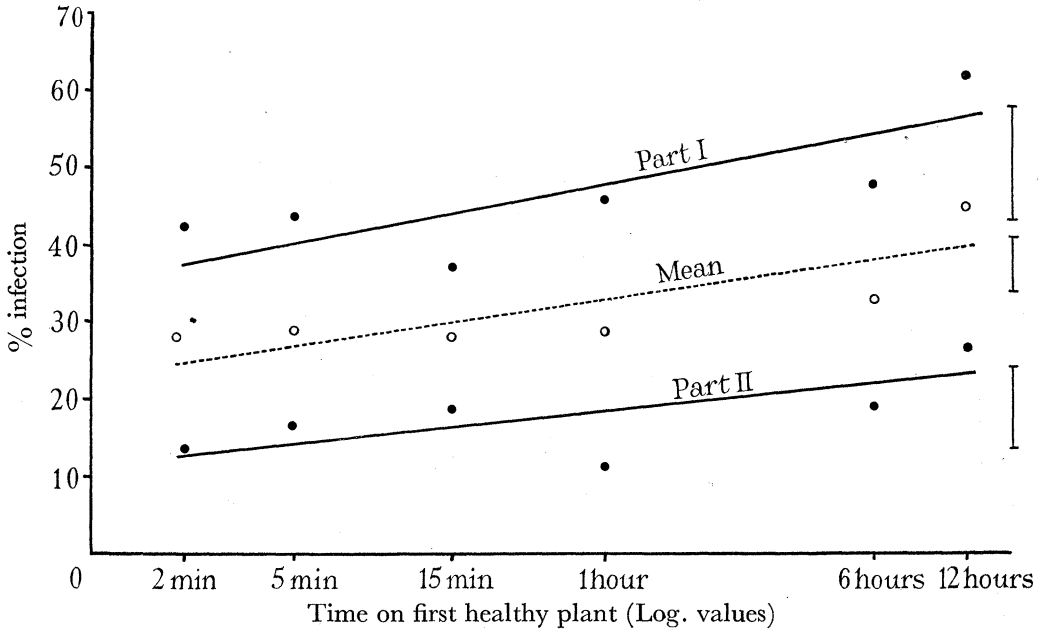

FIG. 7—Effect of varying times on the healthy plant, for all times on the infected plant. Parts I and II of the analysis of variance and their means. Vertical lines = significant differences (S.E. × 3).

There are apparent discrepancies at the ends corresponding to the short feeding times, but these are not significant. The discrepancy in the Part II curve is practically all in the line for 12 hours' feeding on the infected plant. This line shows high percentage values for the points corresponding to the shorter feeding periods, results which do not agree with those shown in figs. 4 and 5.

(*c*) *Interaction between* (*a*) *and* (*b*)—The analysis for Part I shows no significant interaction. For Part II the interaction is significant at the 5% level, but it is difficult to find any immediate explanation for this which would fit in with existing hypotheses. It is probably accounted for by the behaviour of the 12 hours' line which, as has already been pointed out, exhibits unexpectedly high values for the points corresponding to the shorter feeding periods.

There appear to be differences between the rates of increase of percentage infection with time on the healthy plant, corresponding to different feeding times on the infected plant. The regression coefficients are 0·21% for 1 hour's feeding, 0·63% for 6 hours', and 0·12% for 12 hours', but those for 1 hour's and 12 hours' feeding are not significant and they are not significantly different from the 6 hours' regression. On the other hand, two previous experiments, figs. 3 and 4, have shown a significant increase in percentage infection for time on the healthy plant after 12 hours' feeding on the infected plant, so that the present result is probably fortuitous.

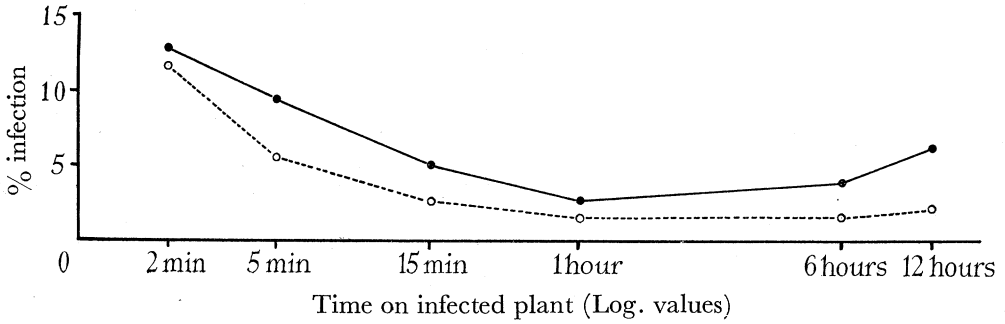

FIG. 8—Consecutive infections. Effect of varying times on the infected plant, for all times on the first healthy plant. • % of second plants infected; ○ % of second plants infected where first plants were also infected.

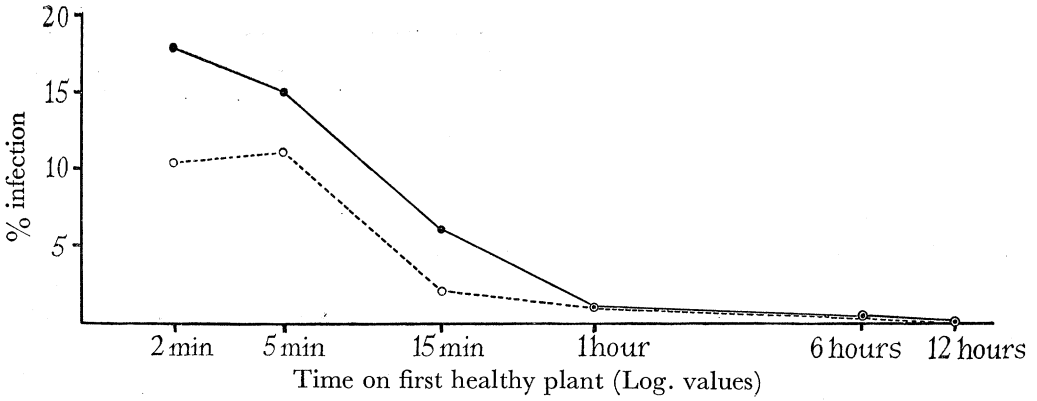

FIG. 9—Consecutive infections. Effect of varying times on the first healthy plant, for all times on the infected plant. • % of second plants infected; ○ % of second plants infected where the first plants were also infected.

(*d*) *Consecutive Infection*—Figs. 8 and 9 show the effect on percentage consecutive infection of times on infected and healthy plants respectively. The solid dots on each graph represent the total percentage of infection from the second feeding on healthy plants (B plants), and the open dots show the percentage of occasions on which both A and B were infected by the same aphid. The actual figures are given in Table XI.

It can be seen from fig. 8 that the curves for varying times on the infected plant have the same form as the corresponding curves for the A feedings (fig. 6), though

with smaller values, showing that there is a close relation between the total quantity of virus disseminated at the first feeding and the amount still active at the second feeding.

The infected and not infected B plants may be grouped in two classes, according to whether the corresponding A plant was infected or not :—

	B not infected	B infected	Total
A not infected	710	26	736
A infected	297	47	344
Total	1007	73	1080

From this arrangement of the data it can be seen that the proportion of second infections amongst aphids which secured a first infection (*i.e.*, $\frac{47}{344} = 0{\cdot}137$) is greater than the proportion of second infections amongst aphids which did not secure a first infection (*i.e.*, $\frac{26}{736} = 0{\cdot}035$). This difference is very highly significant and indicates that there is a real difference between aphids in their ability to transmit the virus. This may be due to variation in the capacity or opportunity for picking up infection from the infected plant, or else in the ability to transmit the infection when obtained.

There are two possible explanations, apart from differences in plant susceptibility, for the failure of certain aphids to infect the first plant on which they are fed though they succeed in infecting the second :—

(*a*) That they did not actually feed on the first plant.

(*b*) That there is variation in individual ability to infect, and that this is partly due to variation in the speed and efficiency with which the virus is transferred to the salivary glands for ejaculation into the plant.

It is not possible to distinguish between these, but consideration of the difference between the total numbers of infections obtained on B plants and those when both A and B were infected (shown as percentages in fig. 9) yields some information.

The numbers of infections of the second healthy plants for different times on the first healthy plant expressed as percentages of their own totals (as these give the best idea of the rate of decrease in percentage infection), are shown in Table XV for two kinds of infections.

TABLE XV

	2 min.	5 min.	15 min.	1 hr.	6 hr.	12 hr.
1. First healthy plant not infected. (Percentage of 26 infections)	50	23	26	0	0	0
2. First healthy plant infected. (Percentage of 47 infections)	40	44	9	4	2	0

If in Class 1 the aphid had always fed on the first healthy plant, the general trend of the figures might be expected to be the same in Class 1 as in Class 2, because in Class 2 it is known that the aphid must have fed on the first healthy plant.

If on the other hand certain of the aphids in Class 1 did not feed on the first healthy plant then the decrease from 2 minutes to 12 hours should be more rapid in Class 1 than in Class 2, because presumably a larger proportion of the aphids will not feed if they are only allowed to remain on the plant for a short time. There is, in fact, little difference in the rate of decrease, though that for Class 1 is if anything slightly steeper, but not sufficiently so to justify the assumption that all the difference is due to non-feeding on the first healthy plant.

The difference between the total number of B plants infected and the number of B plants infected where A was also infected, is smallest after 2 minutes' feeding on the infected plant (fig. 8), and we know from the curves for the A infections (fig. 6) that 2 minutes' feeding gives the highest number of infections on the first healthy plants. If failure to infect the A plant were due to non-feeding on the A plant the number of B infections where A was not infected, should be a constant proportion of the number of A infections, for all times on the infected plant, consequently the greatest number of B infections where A was not infected would be obtained for 2 minutes' feeding.

From these two considerations the influence of non-feeding on the first healthy plant does not appear to be important.

The consecutive infection curve for varying times on the healthy plant (fig. 9) is according to expectation. For 2 and 5 minute periods about 39% of the aphids which gave infection on the first plant, give consecutive infections, but the percentage falls off very rapidly with increasing feeding periods up to 15 minutes, and for longer periods only 3 consecutive infections were obtained out of 540 plants used. The chances of infection of a second healthy plant are therefore very low after more than 15 minutes on the first healthy plant. Within this period the number obtained is relatively high and it is possible that for the very short times more than one consecutive plant might be infected.

IV—Discussion

The present work has shown that the amount of infection obtained in tobacco plants with the virus Hy. III, by means of the aphid *Myzus persicae*, varies in a regular manner according to external conditions. It has been found possible to estimate the probability of infection for different sets of conditions.

Many workers appear to have paid insufficient attention to the numbers of negative infections obtained in experiments. There has even been a tendency to attribute these " failures " to some arbitrarily selected factor or factors such as non-feeding of the aphids (even when 2 hours or more were allowed on suitable plants), or " variation in the ability of the aphids to cause infection ". The assumption is, presumably, that if these factors could be properly controlled, 100% infection would result; but this attitude is due to misconception, and has caused the loss of much valuable information.

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1—*Seasonal Variation*

As the virus agent, the insect vector, the healthy plant, and the infected plant are all concerned in the production of an infection, it is often difficult to distinguish which of them is affected by any particular set of conditions. Nor has it been possible to determine what seasonal climatic factor is responsible for seasonal variation. It seems, however, that of the four variables, the plants concerned are the most likely to be affected by climatic conditions, and of these intensity of illumination or length of day are presumably most important, as temperature and humidity in the glasshouses are partly controlled. The following evidence supports the hypothesis that seasonal variation is due to the effect of light on either the infected or healthy plants, or both, rather than on the aphids.

(1) Seasonal conditions visibly affect the degree of infection, because symptoms are severe, and often lethal in the winter months, but milder in the summer. It is possible that there is a high concentration of virus in the leaves during the winter, and this view is supported by the results of mechanical inoculations.

(2) Furthermore, Samuel, Best, and Bald (1929) have found that pretreatment of healthy tobacco plants in subdued light increased their susceptibility to spotted wilt virus, and I have found that this is also true for Hy. III virus.

(3) The winter maximum percentage infection persisted even when the aphid cultures were receiving artificially increased and lengthened daylight, and therefore, if the effect is one of light, the aphids themselves are not concerned.

2—*Numbers of Aphids*

Given constant or equalized conditions the percentage infection increases in a regular manner with the number of aphids used per healthy plant. Also the infections are local and independent for each aphid and not the result of accumulations of sub-infective doses from different members of a group. This is of importance because it allows each aphid to be considered as an independent source of infection and makes possible the various hypotheses which are put forward to explain other effects.

Since 76% of single aphids are capable of causing infection in optimal conditions (*see* Table X), it is clear that the low numbers of infections which occur in other conditions, when the percentage infection may fall to zero, are not due to an inherent inability of some aphids to cause infection. Storey (1928) has found for Streak disease of Maize that certain strains of the vector *Cicadulina mbila* possessed an inheritable incapacity for infection which was due (Storey, 1933) to some peculiarity of the gut wall, but infectivity trials with several generations of different strains of *Myzus persicae* have given no evidence of consistent inability to infect. Smith (1929) obtained similar results with potato virus Y. Thus, though consistently low percentage infection is obtained with single insect infections for many viruses (*see* Smith, 1933; pp. 181–182), this does not necessarily indicate a fixed low standard of

efficiency in the vectors but is frequently due to fluctuations in their infective capacity which can be increased or decreased according to the conditions of the experiment.

3—*Time on Infected Plant*

The highest proportion of infection is obtained after 2 to 5 minutes' feeding on the infected plant. This might be explained in two ways. (*a*) More virus may be available to the aphid in those areas of the leaf which the stylets reach after two minutes' feeding ; (*b*) the drop in percentage infection with longer feeding periods may be caused by some effect on the virus produced within the insect.

a. Availability of Virus in the Leaf—Bennett (1934) found that the virus of curly top of sugar-beet is confined to the phloem, and cannot be obtained from other tissues of the plant, and therefore it can only be carried by an insect which feeds upon the phloem. Similarly a connexion between other viruses, for example, that of potato leaf-roll, and the phloem tissues, have led to a general suggestion that the success of many vectors may be attributed to the fact of their being phloem feeders.

The rates of penetration of the stylets of *Myzus persicae* into tobacco leaves, similar to those used for the aphid infections, are being investigated and the results so far indicate that more than 5 minutes is required for the stylets to reach the phloem.* Since the percentage infection obtained after 2 minutes on the infected plant is so high it seems probable that Hy. III can be obtained from tissues other than the phloem, and that either the concentration of virus is higher in the superficial tissues than in the phloem, or the subsequent fall in percentage infection is due to an independent action of the aphid on the virus.

b. Effect on the Virus of Conditions Within the Aphid—The aphids are starved for a few hours before feeding on the infected plant (p. 459) and this treatment may have had some effect on the percentage infection obtained from the shorter feeding periods on the infected plant.* Conceivably some of the virus is normally digested and starvation may cause the glandular cells of the stomach wall to enter a resting phase, so that the digestive enzymes are not secreted until a short time after food has entered the mid-gut. This hypothesis, however, assumes that dissemination of the virus into the blood stream occurs in the mid-gut, but the rapidity with which infection can be achieved indicates that dissemination begins as soon as the virus enters the alimentary canal, that is to say, through the extremely thin walls of the oesophagus. The writer has found that when the aphids are fed on intra-vital stains such as eosin, the walls of the oesophagus very quickly become brightly stained, whereas the mid-gut only receives a dilute solution and the dye rarely passes into the hind gut.

In Hoggan's experiments (1933) the aphids were not subjected to preliminary starvation and no effect of feeding time on the infected plant was observed in the results. On the other hand, Hoggan herself points out that her figures for the 5-minute feeding periods are not very reliable. In her experiments the assumption

* Since going to press both these suggestions have been confirmed.

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that some of the aphids were not able to feed in the time allowed is justified, because no allowance was made for "penetration time" which in the conditions of the present experiments is about 5 minutes (p. 474). HOGGAN's figures show a slight drop for percentage infection after one hour's feeding, which might have been more obvious if single aphids had been used over a larger number of trials.

Another hypothesis which can be put forward to explain the high percentage of infection obtained by short feeding times on infected plants is that an antibody to the virus may be formed in the blood of the aphids. The formation of antibodies to various proteins has been described for a number of insects, and CHORINE (1931), using *Galleria mellonella*, finds that immunity can be acquired in from 2 to 7 hours, which agrees with the fact that the aphids appear to be least infective after from 1 to 6 hours' feeding (*see* fig. 6). The fact that infection was not completely suppressed indicates that many of the aphids do not acquire complete immunity, and it would have to be supposed that the antibody is produced at a lower rate than that at which the virus is imbibed. Also, to explain the gradual increase in percentage infection from 1 to 12 hours on the healthy plant, it might be assumed that a maximum rate of formation of antibody is reached, after which it is produced more slowly.

4—*Time on Healthy Plant*

The rise in percentage infection for feeding times on the healthy plants might also be explained on the antibody hypothesis, since MADSEN (1923) has shown that dilution of an antibody-antigen complex, before combination is fully completed, causes dissociation, leading to the production of free antigen, and the imbibing of fresh plant juice might dilute a partially formed antibody-virus complex.

The increase in percentage infection with time on healthy plant might also be explained, if in some aphids the virus circulates more slowly than in others, but this should not influence percentage infection after long periods on the infected plant. Another possibility is that the susceptibility of the plant tissues increases as the result of continuous injury by penetration of the stylets into increasing numbers of cells.

5—*Consecutive Infection*

The fact that aphids are capable of infecting two consecutive healthy plants without intermediate access to a source of infection makes it extremely doubtful that infection is caused by contamination from the outside of the stylets as HOGGAN (1933) and several other workers have suggested. This is in agreement with the conclusions from previous experiments on artificial feeding of *Myzus persicae* (HAMILTON, 1935).

There seems to be some disparity between the consecutive infection results for Hy. III and those of other workers for otherwise similar viruses. HOGGAN (1933) obtained no consecutive infection with cucumber mosaic after from 2 to 48 hours' feeding on the first healthy plant. DOOLITTLE and WALKER (1928) with the same virus state that none occurred after 10 to 20 minutes' feeding, but the actual figures are not

given. Bennett (1932) using red raspberry mosaic and the aphid *Amphorophora rubi*, obtained no consecutive infection after 1 to 20 hours' feeding on the first healthy plant. Consecutive infection obtained after 20 hours he attributed to re-infection of the aphid with virus which had multiplied in the first plant. Smith (1933) gives some evidence to show that *Myzus persicae* can infect a second healthy plant with crinkle Y virus after 24 hours' feeding on the first healthy plant, which may be a similar result to Bennett's; but he gives no information about shorter feeding periods. Whether some of these viruses could be made to give true consecutive infection if shorter feeding periods and larger numbers of plants were used, remains to be proved. Preliminary experiments with Hy. III gave no consecutive infection after 2 hours' feeding on the first healthy plant (p. 19).

6—*Note on Experimental Design*

The accurate estimation of the probability of infection for any given conditions often required much larger numbers of plants than have generally been used. For those viruses in which single aphids give only a small percentage infection, this cannot be measured from 10 or 20 trials (*see* pp. 471, 472). Holme's local lesion method provides a quantitative measure of infection in a single leaf or plant when mechanical inoculation is used, but the local lesion technique has not been found to be practicable for insect infection with Hy. III. Hoggan (1934) has succeeded in obtaining local necrotic lesions with individual aphid infections of Tobacco mosaic in a hybrid of *Nicotiana tabacum* × *N. glutinosa*, and this technique promises to be of value in the further investigation of this virus. The starch lesions obtained in tobacco plants on which *Myzus persicae* in fected with Hy. III virus have fed are often diffuse and very difficult to recognize, and either because of, or in addition to this, smaller numbers were obtained than would have been expected from whole plant infections in the same experimental conditions. This seems to indicate that not all aphids, which cause infection, form local lesions.

Where large numbers of plants have to be used, much economy of time and materials can be effected by employing an efficient experimental design, as in the factorial experiment described in § III, 5. The advantages of such complex experiments have been discussed by Fisher (1935).

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V—Summary

Experiments have been carried out in order to show the effect of various factors on the percentage of infection obtained with the virus Hy. III in tobacco using its insect vector, *Myzus persicae*.

Owing to differences in susceptibility to infection and concentration of the virus between leaves of different ages on the same plant, it is desirable to use leaves of corresponding ages for all aphid feedings in such experiments.

A maximum percentage infection was obtained during the winter months and a minimum during the summer months.

The percentage infection increases with the number of aphids used per plant, and the relation between the numbers of infections obtained for each aphid number indicates that the infections are local and independent.

The percentage infection increases with increased feeding time on the healthy plant, but there is no indication of a preliminary time period in which no infection is obtained.

The percentage infection decreases very rapidly with increasing time on the infected plant from 2 minutes to 1 hour. After 1 hour it increases slightly with further increase of the feeding periods.

The uncertainty as to whether or not aphids have fed on the trial leaves for the exact period allowed, was overcome by either " watched feedings " or by allowing an average " penetration time " of 5 minutes. " Penetration time " was found to be increased by decreasing relative humidity in the insectary.

Myzus persicae is capable of infecting two consecutive plants without intermediate access to a source of infection, but the number of second infections decreases rapidly with increasing time on the healthy plant, and is negligible after 1 hour.

For some aspects of the subject comparisons are made between Hy. III virus and others which appear to be of the same type. Suggestions are made as to the causes of some effects and the mechanisms of infection which are involved.

VI—References

Bennett, C. W. (1932). ' Tech. Bull. Mich. Agric. Exp. Sta. No. 125, East Lansing.'

—— (1934). ' J. Agr. Res.,' vol. **48**, pp. 665–701.

Chorine, A. (1931). ' Bull. biol.,' vol. **65**, pp. 291–393.

Doolittle, S. P., and Walker, M. N. (1928). ' Phytopath.,' vol. **18**, p. 143.

Fisher, R. A. (1935). " The Design of Experiments." Oliver & Boyd, Edinburgh.

Hamilton, M. A. (1932). ' Ann. Appl. Biol.,' vol. **19**, pp. 550–567.

—— (1935). ' Ann. Appl. Biol.,' vol. **22**, pp. 243–258.

Hoggan, I. A. (1933). ' J. Agr. Res.,' vol. **47**, pp. 689–704.

—— (1934). ' J. Agr. Res.,' vol. **49**, pp. 1135–1142.

Holmes, F. O. (1931). ' Contr. Boyce Thompson Inst.,' vol. **3**, pp. 163–172.

Johnson, J., and Hoggan, I. A. (1935). ' Phytopath.,' vol. **25**, pp. 328–343.

Kunkel, L. O. (1926). ' Amer. J. Bot.,' vol. **13**, pp. 646–705.

Madsen (1923). ' J. Sta. Med.,' vol. **31**.

SAMUEL, G., BEST, R. J., and BALD, J. G. (1929). 'Ann. Appl. Biol.,' vol. **22**, pp. 508–524.

SMITH, K. M. (1929). 'Ann. Appl. Biol.,' vol. **16**, pp. 382–399.

———— (1933). "Recent Advances in the Study of Plant Viruses." J. and A. Churchill, London.

STOREY, H. H. (1928). 'Ann. Appl. Biol.,' vol. **15**, pp. 1–25.

———— (1932). 'Proc. Roy. Soc.,' B, vol. **112**, p. 46.

—— (1933). 'Proc. Roy. Soc.,' B, vol. **113**, pp. 463–485.

YOUDEN, W. J., and BEALE, H. P. (1934). 'Contr. Boyce Thompson Inst.,' vol. **6**, pp. 437–454.

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