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The transmission of beet mosaic and beet yellows viruses by aphides; a comparative study of a non-persistent and a persistent virus having host plants and vectors in common

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The vectors of beet mosaic virus are optimally infective when they have fed for only a few minutes on the infected plants after a period of fasting. After infection feeding, infectivity is very rapidly lost when the vectors feed on healthy plants, but while it remains a single vector can infect several plants. Infectivity is lost much more slowly when the vectors fast after infection feeding.

In this behaviour beet mosaic virus resembles Hy 3, potato *Y*, cucumber 1, and other aphis-transmitted viruses which have been called the non-persistent group. It resembles these viruses also in its physical properties.

In some secondary characters beet mosaic differs from the other non-persistent viruses more than they differ from each other. It is retained longer by the fasting vectors, and infectivity of the vectors may increase considerably with increasing infection feeding time, in the absence of preliminary fasting, though it rarely reaches the optimal level.

With beet yellows virus infectivity of the vectors is not affected by preliminary fasting, but always increases with increasing feeding time on both infected and healthy plants. Infectivity increases with increasing feeding time on the healthy plants whatever the infection feeding time, and therefore there is always a delay in the production of optimum infectivity by the aphides after cessation of infection feeding. Infectivity is more rapidly lost from the fasting than from the feeding vectors.

The properties indicate that beet yellows belongs to the persistent group of viruses, although its persistence in the fasting vectors is only about the same as that of beet mosaic, which is a non-persistent virus. The main basis of distinction between the two types seems not to be the time for which they are retained by vectors, but the effect of preliminary fasting.

Beet yellows and beet mosaic viruses have the same vector and host plant and therefore the differences in their behaviour are properties of the viruses themselves, and are not induced by the conditions in which they are transmitted.

In previous papers (Watson & Roberts 1939; Watson 1940), two kinds of insect-transmitted viruses with different vector relationships have been distinguished. These were called 'persistent' and 'non-persistent' viruses, because the most obvious difference between them was the length of time for which the vectors remain infective after ceasing to feed on the infected plants.

Sugar-beet yellows is an example of a persistent virus (Watson 1940). Infectivity of the vector of this virus increases with increasing feeding time on infected plants, and the vectors do not become optimally infective until some time after they have started to feed on the healthy plants. This delay in development of optimum infectivity is independent of the time of feeding on the infected plant. The vectors remain infective for about 72 hr. or less, depending to some extent on the time of feeding on the infected plants. Beet yellows virus is not transmissible mechanically by sap inoculation, but some of its properties have been determined by serological methods (Kleczkowski & Watson 1944); it is inactivated at about 50° C, and remains active in extracted sap at room temperature for about 6 days.

The viruses classed as non-persistent were *Hyoscyamus* virus 3, potato virus *Y*, cucumber virus 1, and tobacco etch virus, all of which were readily transmissible by mechanical inoculation. With these viruses the vectors are most efficient when, after a period of fasting, they are fed for only a few minutes on the infected plants. With longer feeding infectivity decreases. The vectors usually retain infectivity for about 1 hr. when feeding continuously on healthy plants, but longer when they are fasting. These four viruses have similar properties *in vitro*. In extracted sap they are inactivated by keeping for a few days at room temperature, or by 10 min. heating at about 58° C. They are not serologically related to each other.

These non-persistent viruses and beet yellows virus have a common vector in *Myzus persicae* (Sulz.), and the difference in their vector-virus relationships is believed to arise solely from differences in the properties of the viruses. As they have no common host, however, it is possible that the differences might depend to some extent on the host plants. From its general properties, it seemed likely that beet mosaic virus was non-persistent; the experiments described in this paper were made to test this, and to compare and contrast the behaviour of this virus with that of other non-persistent viruses in different host plants, and with beet yellows virus in the same host plant.

Material and methods

The beet mosaic virus used in these experiments was obtained from mangold plants grown on Rothamsted farm. The virus is common in sugar beet and mangold crops in Britain, and it also infects spinach, spinach beet, and some chenopodiaceous weeds. It has been described in this country by K. M. Smith (1937) and is probably the same as the beet mosaic described in the U.S.A. by Robbins (1921). In sugar beet the first symptoms are clearing of the veins of the youngest leaves followed by diffuse mottling over the whole plant, often with dark green areas along the veins,

or minute light green circles on a darker background. The virus is transmissible by sap inoculation, and sugar-beet leaves inoculated by rubbing show no local lesions unless killed, decolorized, and stained with iodine. Longevity *in vitro* (3–4 days), and thermal inactivation point (about 58° C), are similar to those of the non-persistent viruses. It is transmitted by *Myzus persicae* (Sulz.), *Aphis fabae* (Scop.) and *Myzus circumflexus* (Theobald).

The meanings of the special terms used in the text are as follows: infection feeding (I.F.)—the act of feeding on the infected plants; test feeding—feeding on the healthy plants after I.F.; preliminary fasting—a period without food before I.F.; post-infection fasting—a period without food between I.F. and test feeding.

In all the experiments *M. persicae* was used as the vector, and sugar beet as the source of infection and test plant. The methods used in culturing and handling the aphides have been described previously (Watson 1938, 1940). All short feeding periods were timed from the moment of penetration of the leaf by the aphis. The time spent in reaching the feeding position was generally recorded, and it was always insufficient to account for the variations in the behaviour of the aphides.

The factorial designs used in some of the experiments, and the methods of carrying out these and the consecutive infection experiments have also been described previously (*loc. cit.*).

The principle of factorial design is that the treatments tested consist of all combinations of a number of factors at varying intensities. The description of each experiment begins with a list of the factors investigated, giving the variants of each. The experiments were mainly concerned with the same factors, but one or more of the factors were held constant in each experiment; these are also given in the descriptions. Each experiment consisted of a number of replications, carried out on different dates, in which each treatment was applied to the same number of plants. Therefore the number of plants used for each combination of treatments is the number of plants used in each replication multiplied by the number of replications; e.g. in experiment 1, 5 plants per treatment × 6 replications gives 30 plants for each combination of treatments. The figures given in the tables are the numbers of plants infected out of the total number used for each combination of treatments.

The experiments are grouped in four sections according to the general treatment of the aphides.

I. Effect of varying preliminary fasting and infection feeding times

Experiment 1 (*table* 1). *Beet mosaic virus*

Treatments—all combinations of:

(1) Three preliminary fasting times: 0, 1, 20 hr.

(2) Four infection feeding times: 2, 15 min., 1, 20 hr.

Constant: test feeding time, 24 hr.

Aphides: 1 per plant.

Plants: 5 per treatment, 6 replications.

TABLE 1. THE EFFECT OF VARYING TIMES OF PRELIMINARY FASTING AND INFECTION FEEDING ON THE TRANSMISSION OF BEET MOSAIC VIRUS BY *M. PERSICAE*

preliminary fasting times	infection feeding time				
	2 min.	15 min.	1 hr.	20 hr.	total
0	2	4	2	6	14 (*a*)
1 hr.	15	3	3	10	31
20 hr.	15	6	4	12	37
total	32	13	9	28 (*b*)	82

Standard errors (*a*) ± 3·07, (*b*) ± 2·66.

The number of plants infected with beet mosaic virus was greatly increased by 1 hr. preliminary fasting, but further increase after an additional 19 hr. was only slight. Aphides which fasted before I.F. gave about 65 % fewer infections after 1 hr. I.F. than after 2 min., but their capacity to cause infection increased again between 1 and 20 hr. I.F. When there was no preliminary fasting the effect of increase of I.F. time was small.

Experiment 2 contrasts the behaviour of beet mosaic and beet yellows viruses in response to varying fasting and feeding conditions. The first part, experiment 2*a*, shows the effect of varying I.F. time with preliminary fasting constant; in the second part, experiment 2*b*, both I.F. and preliminary fasting times are varied.

Experiment 2 (*table* 2). *Beet mosaic and beet yellows viruses*

(*a*) Treatments—all combinations of:

(1) Beet mosaic and beet yellows viruses.

(2) Three infection feeding times: 3, 30 min., 24 hr.

Constant: preliminary fasting time, 4 hr.; test feeding time, 24 hr.

Aphides: mosaic 1 per plant, yellows 5 per plant.

Plants: 10 per treatment, 4 replications.

(*b*) Treatments—all combinations of:

(1) Beet mosaic and beet yellows viruses.

(2) Two preliminary fasting times: 0, 16 hr.

(3) Four infection feeding times: 3 min., 1, 5, 20 hr.

Constant: test feeding time, 24 hr.

Aphides: mosaic 1 per plant, yellows 5 per plant.

Plants: 7 per treatment, 4 replications.

In experiment 2 beet mosaic virus behaved as in experiment 1. The greatest number of plants was infected by aphides which had received preliminary fasting and short I.F.; the number decreased with longer I.F. times up to 1 hr., and then increased again between 1 and 24 hr. I.F. When there had been no preliminary fasting few plants were infected after 2 min. I.F., but the number after 24 hr. I.F. was the same as when the aphides had fasted before I.F.

TABLE 2. THE EFFECT OF PRELIMINARY FASTING AND VARYING INFECTION FEEDING TIMES ON THE INFECTIVITY OF *M. PERSICAE* COMPARED FOR BEET YELLOWS AND BEET MOSAIC VIRUSES

			infection feeding times after 4 hr. preliminary fasting			
			5 min.	30 min.	24 hr.	total
(*a*)		B.Y.V.	1	3	18	22
		B.M.V.	17	5	8	30

		preliminary fasting times	infection feeding times				
			5 min.	1 hr.	5 hr.	24 hr.	total
(*b*)	B.Y.V.	none	1 (*a*)	5	16	25	47 (*b*)
		16 hr.	1	6	20	24	51
	B.M.V.	none	6	7	11	15	39
		16 hr.	21	6	11	17	55

Standard errors (*a*) ± 2·96, (*b*) ± 5·92.

With beet yellows virus preliminary fasting had no effect in varying the time at which optimum infectivity occurred. Whether the aphides fasted or not, the number of plants infected after a short I.F. time was negligible. Preliminary fasting did not affect either the rate of increase or the level of infectivity which was eventually reached.

About the same numbers of plants became infected with beet mosaic and beet yellows viruses, but the mosaic virus was transmitted by 1 and the yellows virus by 5 aphides per plant. In these conditions, therefore, *M. persicae* is a more successful vector of beet mosaic than of beet yellows virus, at least when the capacity of the aphides is determined from single and not from consecutive test feedings.

The fact that the transmission of beet mosaic virus was greatly affected by preliminary fasting and that of yellows virus was not, shows that the effect could not have been directly one of 'appetite', that is, increased rate of feeding induced by starvation. Obviously some other factor is involved in the effect of preliminary fasting.

II. CONSECUTIVE INFECTION EXPERIMENTS

Experiments 3 and 4 were made to test the effect of varying fasting and feeding treatments on the capacity of the aphides to transmit the viruses to several healthy plants in succession after a single I.F.

Experiment 3 (*table* 3). *Beet mosaic virus*

Treatments—all combinations of:

(1) Two infection feeding times: 3 min., 20 hr. (trials 1 and 2).

(2) Two test feeding times on successive plants: 5, 50 min. (trial 1 only).

Constant: preliminary fasting time, 16 hr.

Note. Each aphis fed for the same test feeding time on the first five successive plants and for 16 hr. on the last plant.

TABLE 3. NUMBER AND DISTRIBUTION OF SUCCESSIVE INFECTIONS CAUSED BY *M. PERSICAE* TRANSMITTING BEET MOSAIC VIRUS

N.B. In trial 1, thirty-six aphides were tested for each test feeding time, and in trial 2, made in short test feeding times only, twenty aphides were tested. Aphides which failed to give infection are not included in the table.

Performance at successive 5 min. test feeding periods

	3 min. infection feeding							20 hr. infection feeding						
Successive plants ...	1	2	3	4	5	6	frequency*	1	2	3	4	5	6	frequency
trial 1	+	0	0	0	0	0	5	+	0	0	0	0	0	3
	+	+	0	0	0	0	1	+	+	+	+	0	0	1
	+	0	+	0	0	0	1	0	+	0	0	0	0	2
	+	+	0	+	0	0	1	0	0	+	+	0	0	1
	+	+	+	+	0	0	1	0	0	0	0	+	0	1
	+	+	0	0	0	+	1							
	+	+	+	0	0	0	1							
	0	+	+	0	0	0	1							
Infections on successive plants	11	6	4	2	0	1	total 24	4	3	2	2	1	0	total 12
Total number of aphides infective							12	.....						8
trial 2	+	0	0	0	0		4	+	0	0	0	0		3
	+	+	0	0	0		3	0	+	0	0	0		2
	+	0	+	0	0		1	0	0	+	0	0		1
	+	+	+	0	0		1	0	+	+	0	0		1
								0	0	0	+	0		1
Infections on successive plants	9	4	2	0	0		total 15	3	3	2	1	0		total 9
Total number of aphides infective							9	.....						8

Performance at successive 50 min. test feeding periods

trial 1	+	0	0	0	0	0	11	+	0	0	0	0	0	9
								0	+	0	0	0	0	1
Infections on successive plants	11	0	0	0	0	0	total 11	9	1	0	0	0	0	total 10
Total number of aphides infective							11	.....						10

* Columns headed 'frequency' give the number of times each distribution occurred.

There are two aspects of these experiments to be considered. One is the effect of the treatments on the ability of individual aphides to cause consecutive infections, and the other is their effect on the total numbers of plants infected, i.e. the efficiency of the aphides considered as a group. Thus varying the time of test feeding had little effect on the number of aphides which became infective, but the ability of the aphides to cause consecutive infections was greatly affected. When the test feeding time was 50 min. (trial 1), twenty-one aphides caused infection but they infected only one plant each; when the test feeding time was 5 min., twenty aphides caused infection, but half of them infected more than one plant.

Varying the I.F. time also had comparatively little effect on the number of aphides which became infective. Aphides given 20 hr. I.F. infected slightly fewer plants than those given 3 min.; no 1 hr. I.F. treatment was used, so that the aphides were not tested in conditions of minimum vector efficiency. However, when the test feeding time was 5 min., varying the I.F. period did affect the capacity of the aphides to cause consecutive infections. The twenty-one aphides which became infected after 3 min. I.F. in trials 1 and 2 together infected a total of thirty-nine plants, but the sixteen which became infective after 20 hr. I.F. infected only twenty-one plants.

After preliminary fasting and short I.F. time, most of the aphides caused infection during the first 5 min. of feeding if they were going to infect at all. Therefore the vector efficiency of the aphides as a group did not increase with increasing total test feeding time, that is, with the increasing sum of all the times spent on the successive plants. After a long I.F. time, during which the influence of preliminary fasting had been lost, many of the aphides fed on one or more consecutive plants before causing infection, and there were more aphides which had caused infection by the end of the experiment than there were at the beginning. Thus the number of aphides which caused infections increased with increasing total test feeding time.

With Hy 3 virus (Watson 1936) a slight increase in infectivity of the vectors with increasing test feeding time was observed when there had been no preliminary fasting. In later experiments, when preliminary fasting treatments were used, there was no such increase.

Table 4 shows the performance of vectors of Hy 3, potato *Y*, and severe etch viruses, in consecutive 5 min. test feedings after preliminary fasting and 2 or 3 min. I.F. periods, compared with results for beet mosaic virus obtained in similar fasting and feeding conditions (experiment 3, trial 1).

TABLE 4. NUMBERS OF SUCCESSIVE PLANTS INFECTED BY *M. PERSICAE* IN 5 MIN. FEEDINGS GIVEN AFTER PRELIMINARY FASTING AND SHORT INFECTION FEEDING. COMPARED FOR BEET MOSAIC, HYOSCYAMUS 3, POTATO *Y* AND TOBACCO ETCH VIRUSES

Figures in brackets show the number of aphides used in each experiment, at the rate of 1 per plant.

		successive healthy plants									
		1	2	3	4	5	6	7	8	9	10
beet mosaic virus	(36)	11	6	4	2	0	1	—	—	—	—
Hyoscyamus 3 virus*	(36)	23	12	10	9	8	6	3	1	1	4
potato *Y* virus*	(24)	13	11	6	6	3	1	2	2	—	—
tobacco etch virus†	(30)	18	8	5	2	8	—	—	—	—	—

* Watson & Roberts (1940). † Kassanis (1941).

Table 4 shows that *M. persicae* was a less successful vector of beet mosaic than of the other viruses, but otherwise the results were similar. With all the viruses, after preliminary fasting and short I.F. time, most of the vectors infected the first

plants on which they fed and subsequent infections were all caused by these aphides, so that the number of aphides which caused infection did not increase with total test feeding time.

The rate of loss of infectivity from the aphides while feeding seemed to be about the same for all the viruses, but initial infectivity of beet mosaic virus was low and was exhausted on the first five or six plants. With the other viruses infectivity was retained longer, and also its rate of loss became reduced after the first five or six consecutive feedings. At this time individual aphides were weakly infective; they could cause only one, or rarely two, infections among the last four plants on which they were fed, but these infections were produced after longer intervals of feeding than at first, so that the infectivity of the group was reduced less rapidly. Also, more of the last plants, on which the aphides were fed for about 20 hr., became infected than those immediately preceding them, which confirms that the infectivity of the aphides was then being influenced by the test feeding time. In this, the behaviour of aphides after preliminary fasting and short I.F. at the end of a series of consecutive test feedings, resembled that of aphides transmitting beet mosaic virus in short consecutive test feedings after long I.F., and the determining factor seems to be the length of time which has elapsed since the preliminary fasting treatment.

To sum up, it seems that with non-persistent viruses after preliminary fasting and short I.F. periods aphides contain much active virus, but lose it very rapidly. When the influence of fasting before I.F. is nullified by a further period of feeding on either infected or healthy plants, the aphides contain very much less active virus, but it is lost more slowly.

Experiment 4 was made to determine the distribution of infection obtained with beet yellows virus in successive test feedings in order to compare it with that of beet mosaic virus. The time scale over which changes in the performance of the aphides take place with the two viruses is so different that they could not satisfactorily be compared in the same experiment. With beet yellows virus 1 hr. test feeding causes about the same proportional change in the performance of aphides as 5 min. feeding with beet mosaic virus, and this seemed to be the best basis of comparison.

Experiment 4 (*table* 5). *Beet yellows virus*

Treatments—constant.

Infection feeding time: 24 hr.

Test feeding time: 1 hr. on five successive plants; 16 hr. on the 6th plant, and 1 hr. on the 7th and 8th.

Aphides: 1 per plant.

Plants: 10 sets of 8 successive plants; 7 replications.

The distribution of infection with beet yellows virus resembled that of beet mosaic virus after long I.F., in that the aphides developed infectivity after varying times from the beginning of the consecutive test feedings; but once the aphides had infected a plant they generally infected several more, so that the total number of

plants infected at each transfer increased for the first 2 or 3 hr. With beet mosaic virus, although there is an increase of infectivity with increasing feeding time, the aphides are only weakly infective, and rarely infect more than one plant in succession, so that the number of plants infected does not increase, although the number of aphides exhibiting infectivity does so.

TABLE 5. NUMBER AND DISTRIBUTION OF SUCCESSIVE INFECTIONS CAUSED BY *M. PERSICAE* IN TRANSMITTING BEET YELLOWS VIRUS

Performance at successive 1 hr. test feeding periods.

plants ...	1	2	3	4	5	6	(16 hr.)	7	8
	+	0	0	0	0	0		0	0
	+	+	0	0	0	0		0	0
	+	+	0	0	+	0		0	−
	+	+	+	0	0	+		+	0
	+	0	+	0	+	0		0	+
	+	0	0	+	0	+		−	−
	0	+	0	0	0	0		0	0
	0	+	0	+	0	+		0	0
	0	+	0	+	0	+		0	−
	0	+	+	0	0	+		0	+
	0	+	+	+	0	0		0	0
	0	0	+	0	+	0		+	0
	0	0	+	0	0	+		0	0
	0	0	+	0	0	+		0	0
	0	0	+	+	+	+		+	0
	0	0	+	+	0	0		0	0
	0	0	0	+	0	+		−	−
	0	0	0	0	0	+		+	+
total	6	8	9	7	4	10		4	3

− aphis died.

Fifty-two aphides failed to cause infection on any plant.

With beet yellows virus the increase in the number of aphides to exhibit infectivity continued for the first 6 hr. of consecutive feeding, but the number of plants infected at each transfer decreased after the first 3 hr. because the aphides which first became infective ceased to infect, or infected at longer and longer intervals. Infectivity was higher again at the sixth transfer, as with the non-persistent viruses on the last of their consecutive plants, because the aphides were given longer test feeding time.

Had short I.F. times been given it is obvious that the distribution of infection with beet yellows virus would have been entirely different from that obtained with beet mosaic and the non-persistent viruses; preliminary fasting does not affect vector efficiency with beet yellows virus (see p. 204), and the effect of short I.F. on the infectivity of non-persistent viruses depends upon the preliminary fasting treatment.

When the effect of preliminary fasting is not involved there seems, superficially, to be no essential difference between the transmission of beet yellows and beet mosaic

virus, except in the time taken to acquire and disseminate infection and the number of plants infected. When there has been preliminary fasting the transmission of the two types of virus is entirely different. Thus one of the essential differences between them lies in the special property which makes beet mosaic and the other non-persistent viruses susceptible to the effect of preliminary fasting.

III. Effect of post-infection fasting

With Hy 3, potato *Y* and tobacco etch viruses, infectivity was retained longer by the vectors when fasting after infection feeding than when feeding, and the optimum retention of infectivity during post-infection fasting was by aphides which had received preliminary fasting and short I.F. Experiment 5 shows the effect of these treatments on the transmission of beet mosaic virus.

Experiment 5 (*table* 6). *Beet mosaic virus*

Treatments—all combinations of:

(1) Two preliminary fasting times: 0, 4 hr.

(2) Three infection feeding times: 2 min., 1, 16 hr.

(3) Four post-infection fasting times: 0, 1, 5, 20 hr.

Constant: test feeding time, 20 hr.

Aphides: 1 per plant.

Plants: 5 per treatment, 4 replications.

Table 6. Effect of varying times of preliminary fasting, infection feeding and post-infection fasting on transmission of beet mosaic virus by *M. persicae*

preliminary fasting times	infection feeding times	post-infection fasting times				
		0	1 hr.	5 hr.	20 hr.	total
none	2 min.	6	3	2	1	12 (*b*)
	1 hr.	3	5	1	0	9
	16 hr.	16	10	8	3	37
	total	25 (*a*)	18	11	4	58 (*d*)
4 hr.	2 min.	18	7	2	4	31 (*b*)
	1 hr.	7	4	3	1	15
	16 hr.	17	10	10	6	43
	total	42 (*a*)	21	15	11	89 (*d*)
	total	67 (*c*)	39	26	15	147

Standard errors: (*a*) ± 1·77, (*b*) ± 2·05, (*c*) ± 2·51, (*d*) ± 3·55.

The effects of varying preliminary fasting and I.F. periods were the same as in experiments 1 and 2. As in experiment 2 the number of plants infected increased with increasing I.F. for both times of preliminary fasting. The infections decreased with increasing post-infection fasting time, and the rate of decrease was most rapid for the first few hours of fasting.

Beet mosaic virus was retained by the fasting vectors longer than the other non-persistent viruses. 22 % of the initially infective aphides were still infective after 20 hr. fasting. Previously Hy 3 had survived in the fasting vector longest of the non-persistent viruses, but less than 3 % of the aphides retained infectivity for even 12 hr. (Watson 1938). This compares very unfavourably with the performance of the same vectors with beet mosaic virus.

Beet yellows virus survives in the feeding vectors for several days, and it might be supposed that it would be lost even more slowly from the fasting vectors, since feeding vectors presumably lose virus by ejaculation into the healthy plants. Experiments 6 and 7 show that this is not so.

Experiment 6 (*table* 7). *Beet yellows virus*

Treatments—all combinations of:

(1) Three infection feeding times: ½, 3, 20 hr.
(2) Three post-infection fasting times: 0, 3, 20 hr.
(3) Three test feeding times: ½, 3, 20 hr.

Aphides: 5 per plant.

Plants: 5 per treatment, 8 replications.

TABLE 7. EFFECT OF POST-INFECTION FASTING WITH VARYING INFECTION AND TEST FEEDING TIMES. BEET YELLOWS VIRUS

test feeding times	infection feeding times	post-infection fasting times			
		0	3 hr.	20 hr.	total
½ hr.	½ hr.	4 (*a*)	1	5	10 (*b*)
	3 hr.	13	14	6	33
	20 hr.	23	12	7	42
	total	40 (*b*)	27	18	85 (*c*)
3 hr.	½ hr.	5	8	3	16
	3 hr.	33	18	8	59
	20 hr.	34	24	16	74
	total	72	50	27	149
20 hr.	½ hr.	7	3	4	14
	3 hr.	24	19	9	52
	20 hr.	35	28	11	74
	total	66	50	24	140

Standard errors: (*a*) ±3·13, (*b*) ±5·42, (*c*) ±9·39.

The number of plants infected increased with increasing I.F. and test feeding times as in previous experiments. Infectivity was lost unexpectedly rapidly during post-infection fasting. Infectivity decreased by 34 % during the first 3 hr. of fasting, although if the aphides had fed on healthy plants for 3 hr. their infectivity at a subsequent feeding would have been higher than their initial infectivity.

There was no indication that when the aphides were allowed to feed again after fasting they recovered any of the infectivity which they had lost, for the difference between the performances of aphides which had fasted and those which had not fasted was about the same for 30 min. test feeding as for 20 hr. If the aphides recovered infectivity during feeding on the test plants, the decrease in infectivity with increasing post-infection fasting time would have been smaller for 20 hr. test feeding than for 30 min.

The decrease in the number of plants infected caused by increasing the post-infection fasting time was slower when the I.F. time was short than when it was long. This appeared in the analysis of variance as a highly significant interaction between I.F. and post-infection fasting time. Thus the rate of loss of infectivity during fasting seems to depend to some extent on the aphides' initial content of active virus, and is slower when this quantity is small than when it is large.

Experiment 7 was made with beet yellows virus to compare directly the effect of post-infection fasting with that of feeding on intermediate healthy plants.

Experiment 7 (*table* 8). *Beet yellows virus*

Treatments—all combinations of:

(1) Post-infection fasting and feeding on intermediate healthy leaves.

(2) Six times of post-infection fasting or intermediate feeding: 0, 1, 2, 5, 10, 20 hr.

Constant: I.F. time 24 hr.

Test feeding time: 2 hr.

Aphides: 3 per plant.

Plants: 10 per treatment, 7 replications.

TABLE 8. COMPARISON OF EFFECTS OF POST-INFECTION FASTING AND FEEDING ON INTERMEDIATE HEALTHY PLANTS, FOR VARYING TIMES. BEET YELLOWS VIRUS

treatment of aphides after I.F.*	times of feeding or fasting						
	0	1 hr.	2 hr.	5 hr.	10 hr.	20 hr.	total
feeding	33 (*a*)	37	44	32	18	13	177 (*b*)
fasting	31	27	23	14	10	7	112

Standard errors: (*a*) ±4·66, (*b*) ±7·98.

* Test feeding time = 2 hr.

The number of plants infected decreased rapidly during the first 5 hr. of post-infection fasting, but there was no loss of infectivity during the corresponding period of feeding on intermediate healthy leaves. Instead, more plants were infected after 2 hr. of intermediate feeding than after 0 or 1 hr., and after 5 hr. intermediate feeding the aphides still maintained their initial level of infectivity. This has also been found to occur when the intermediate feeding period was spent on *Hyoscyamus niger* which is not susceptible to beet yellows virus; so that the maintenance of the infectivity of the aphides is due merely to the fact that they

have been feeding and not to reinfection, or to the virus being kept active in a medium consisting of susceptible plant sap. After the first 5 hr. of treatment the rate of loss of infectivity from fasting aphides became slower, and that from the feeding aphides faster, so that eventually the rates of loss were about the same. It is known that feeding aphides would continue to lose infectivity slowly for about another 48 hr. (Watson 1940), but it would not be practicable to test the loss from fasting aphides over this period as their general condition would deteriorate and they would not be comparable with the feeding aphides.

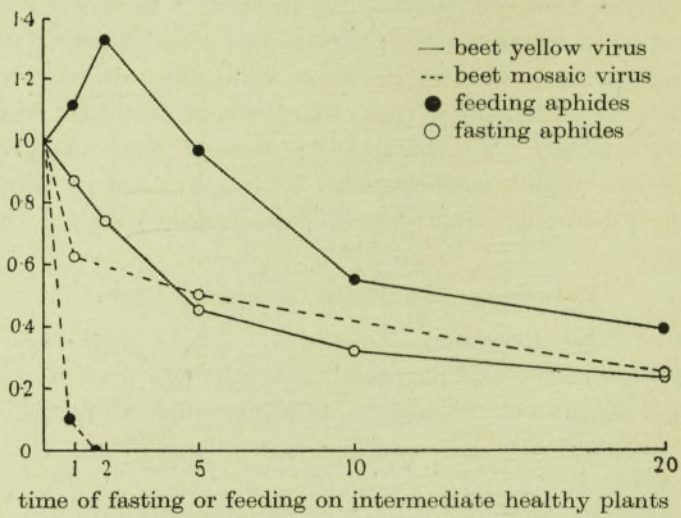

FIGURE 1. Changes in the ability of *M. persicae* to transmit beet yellows and beet mosaic viruses, after varying feeding and fasting treatments. The solid lines refer to beet yellows and the dotted lines to beet mosaic virus. The points marked by solid circles show the infectivity of aphides which fed between I.F. and test feeding, and the open circles show the infectivity of aphides which fasted between I.F. and test feeding.

Beet yellows virus thus differs greatly from beet mosaic and the other non-persistent viruses in that the number of plants infected decreases more rapidly when the vectors fast than when they feed; with all the non-persistent viruses tested, infectivity is lost much more rapidly by the feeding than by the fasting vectors. This difference in behaviour in response to treatment of the vector is shown graphically in figure 1, for beet mosaic and beet yellows viruses. The data used are taken from tables given in this paper.

In figure 1 the infectivity of the aphides after varying post-infection fasting and feeding treatments is expressed as a fraction of their infectivity immediately after cessation of I.F. The I.F. times were 16–24 hr., and the test feeding times were 2 hr. for the beet yellows experiments; 50 min. for the beet mosaic vectors which were given post-infection feeding, and 20 hr. for those given post-infection fasting, as no short test feeding figures were available for this treatment.

The behaviour of the two viruses in the feeding aphides is very different. Infectivity with beet mosaic virus was lost during the first hour of feeding, while that

with beet yellows virus was only reduced by 50 % during 20 hr. of feeding. In the fasting aphides, however, their behaviour was similar, for both retained about 20 % of their infectivity after 20 hr. fasting. The loss from the fasting vectors of beet mosaic during the first hour of fasting was more rapid than that of beet yellows.

The behaviour of beet mosaic virus during post-infection fasting is more consistent than that of beet yellows virus. In experiment 6, the changes in infectivity due to the varying treatments were about the same on each occasion, hence the smallness of the errors. In experiment 7 with beet yellows virus, and also in experiments 8 and 9, the losses during the first few hours of fasting were very variable, sometimes large, and sometimes inconsiderable, hence the errors were much larger in these experiments than in the beet mosaic virus experiments. It is possible that the variation in rate of loss of beet yellows virus from the vectors was due to variations in glasshouse temperatures. Kassanis (1941) showed that loss of severe etch virus from the fasting vectors was much slower when the insects were held at low temperatures, and beet yellows virus is more sensitive to changes in temperature than any of the non-persistent viruses (Kleczkowski & Watson 1944).

Experiment 8 shows that a period of feeding given before the post-infection fasting treatment does not prevent rapid loss of infectivity during subsequent fasting; i.e. the introduction of healthy sap into the vectors does not prevent their losing infectivity unless feeding is maintained. Also there is no recovery of the infectivity lost during fasting, detectable in two 1½ hr. feeding periods given after the fasting treatment.

Experiment 8 (*table* 9). *Beet yellows virus*

Treatments—all combinations of:

(1) Aphides fed for 1½ hr. on healthy leaves immediately after I.F. (before post-infection treatment), and aphides not fed after I.F.

(2) 12 hr. post-infection fasting and 12 hr. intermediate feeding.

Constant: I.F. time, 24 hr.; two consecutive test feedings of 1½ hr. each.

Aphides: 2 per plant.

Plants: 10 per treatment, 5 replications.

TABLE 9. EFFECT OF CONSECUTIVE FEEDING AFTER POST-INFECTION FASTING, AND OF FEEDING ON HEALTHY PLANTS BEFORE POST-INFECTION FASTING

		infections obtained at two successive 1½ hr. test feedings		
treatments		A	B	total
post-infection fasting—12 hr.	1½ hr. feeding between I.F. and fast	11	12	23
	no feeding between I.F. and fast	17	8	25
	total	28	20	48
intermediate feeding—12 hr.	1½ hr. feeding between I.F. and intermediate feeding	19	18	37
	no feeding between I.F. and intermediate feeding	20	19	39
	total	39	37	76

Fewer plants were infected when the aphides fasted before test feeding than when they fed; a period of feeding given before the post-infection fasting or feeding treatment slightly reduced the number of plants infected, but did not affect the loss due to fasting. As there was no increase in infectivity between the first and second successive test feedings given after the fasting or feeding treatments, the long fasting period did not merely delay the increase in infectivity of the aphides which normally occurs during the first few hours of test feeding, but eliminated it.

IV. The separation of beet yellows and beet mosaic viruses from a mixed infection in the same host plant, by differential fasting and feeding treatments of the vectors

Viruses with the same vectors and host range are sometimes difficult to isolate from the complexes in which they occur in nature. Experiment 9 shows the use of differential fasting and feeding treatments in separating such complexes, and it also shows that the behaviour of the viruses is substantially the same with *A. fabae* (Scop.) as the vector, as it is with *M. persicae* (Sulz.), although *A. fabae* is a less successful vector. The sources of infection were young sugar-beet plants infected by aphides at the same time with beet mosaic and beet yellows viruses.

Experiment 9 (*table* 10). *Beet mosaic and beet yellows viruses vectors* M. persicae *and* A. fabae

Treatments—all combinations of:

(1) Vectors: *M. persicae* and *A. fabae*.

(2) Two preliminary fasting times: 0, 16 hr.

(3) Three infection feeding times: 5 min., 1, 24 hr.

Constant: Four consecutive test feeding times, A 10 min., B 2 hr., C 24 hr., D 24 hr.

Aphides: 2 per plant.

Plants: 5 per treatment, 6 replications.

The separation of the viruses was very successfully accomplished, as only three plants became infected with both viruses at once. With beet mosaic virus, both *M. persicae* and *A. fabae* caused an optimum number of infections after preliminary fasting and short I.F. These infections occurred during the first 10 min. of feeding on the consecutive healthy plants. A second optimum occurred after 24 hr. I.F., and most of the infections with beet mosaic virus were again on the first plants. With beet yellows virus very few plants became infected when the I.F. time was short; after 24 hr. I.F. a few of the second and many of the third plants became infected. Beet mosaic was never carried to the third plants, but after 24 hr. I.F. a few of the second plants became infected, and mixed infections occurred.

In practice the separation of unknown viruses from a complex does not require so elaborate a technique as that used in experiment 9, which was designed to show

how the method works, and to compare the performance of the two aphides, as well as to separate the viruses. The only treatment necessary would be preliminary fasting of 12–16 hr. (overnight), and two infection feeding times—2–5 min. and 24 hr.; followed by consecutive feedings of which the first must be short (about 10 min.), and the second 2 or 3 hr., in order to eliminate any non-persistent virus from the aphides. The length of the later consecutive feedings would depend upon the viruses to be isolated, for the period of delay in production of optimum infectivity during test feeding time varies very much with different persistent viruses. For instance, with beet yellows virus it is only a few hours, but with other persistent viruses it may be many hours, or even days, before the optimum number of infections is obtained, and with these viruses infectivity of the vectors is at first absent or negligible. After some hours' feeding, vector efficiency with a virus like beet yellows is much reduced, and the virus with the longer period of delay in the production of optimum infectivity would be obtained alone.

TABLE 10. SEPARATION OF BEET MOSAIC AND BEET YELLOWS FROM A MIXED INFECTION, BY DIFFERENTIAL TREATMENT OF THE VECTORS

infection feeding time	vector	virus	no preliminary fasting consecutive infections A	B	C	D	total	16 hr. preliminary fasting consecutive infections A	B	C	D	total
5 min.	*M. persicae*	mosaic	3	1	0	0	4	15	2	0	0	17
		yellows	0	0	1	0	1	0	0	0	0	0
	A. fabae	mosaic	1	0	0	0	1	3	1	0	0	4
		yellows	0	0	0	0	0	0	0	0	0	0
1 hr.	*M. persicae*	mosaic	1	0	0	0	1	2	1	0	0	3
		yellows	1	3	1	0	5	0	2	1	0	3
	A. fabae	mosaic	0	0	0	0	0	0	0	0	0	0
		yellows	0	1	0	0	1	1	0	0	0	1
24 hr.	*M. persicae*	mosaic	4	1*	0	0	5	6*	2*	0	0	8
		yellows	2	16	17	6	41	4	18	22	6	50
	A. fabae	mosaic	0	0	0	0	0	1	0	0	0	1
		yellows	0	3	4	1	8	0	5	5	2	12

Consecutive feeding times: A, 10 min.; B, 2 hr.; C, 24 hr.; D, 24 hr.

* The figures for mosaic and yellows are determined from the same set of plants. At * one plant became infected with both viruses and therefore appears as both mosaic and yellows.

Probably the most effective treatment would be to give two 24 hr. periods for the third and fourth consecutive feedings, as was done in experiment 8; then, if the infection obtained were still suspected of being a complex, to use this isolate in further consecutive experiments giving three or four longer or shorter consecutive feeding periods, according to where the infection was obtained in the previous experiment.

Thus the method could be used for separating different persistent viruses from each other as well as persistent from non-persistent viruses, though it would not

necessarily be successful in separating non-persistent viruses from each other, as their behaviour in the feeding vectors is too similar. They might be separable by differential post-infection fasting treatments of the aphides.

Discussion

When the aphides transmitting beet mosaic virus are made to fast before feeding on a source of infection they infect the greatest number of plants if they are given only 2 or 3 min. I.F. Their infectivity decreases by about 60 % during the next hour of I.F. and increases again, sometimes to its initial level, when the I.F. time is increased to several hours. When there has been no preliminary fasting the infectivity of the aphides remains constant at a low level for the first 2 or 3 hr. I.F., and then increases at about the same rate as it does with aphides which have fasted before I.F.

After preliminary fasting and short I.F. a vector can transmit beet mosaic virus to several healthy plants in successive 5 min. test feedings, but the probability of infection decreases at each transfer, and infectivity of the aphides is exhausted after about the sixth transfer, that is, about 35 min. from the cessation of I.F. In these conditions most of the aphides infect the first plants on which they feed. When the aphides are given several hours' I.F. fewer of them become infective, and fewer plants are infected by each individual. The infectivity of the aphides as a group, however, declines less rapidly than it does after preliminary fasting and short I.F., because some of them do not infect the first plants on which they feed but become infective at progressively later feedings. In other words, the number of aphides exhibiting infectivity increases with increasing time of feeding on healthy plants, although the total number of plants infected still decreases at each successive transfer.

When aphides fast after I.F. infectivity is lost much more slowly than when they feed on healthy plants. After fasting for 20 hr. vectors of beet mosaic virus retained, on the average of all previous treatments (preliminary fasting and infection feeding times), about 20 % of their initial infectivity. The loss of infectivity from aphides given 1 hr. I.F. was slightly more rapid during fasting than when they had been given very short or very long I.F. time.

On the whole this behaviour of beet mosaic virus in relation to its vectors accords with that of the sap-transmissible, aphid-transmitted viruses which have been called the non-persistent group. The other viruses which have been found to exhibit these properties are: *Hyoscyamus* 3, potato *Y*, cucumber 1, severe etch of tobacco, soya bean mosaic, and common pea mosaic.

The ability of vectors to transmit beet yellows virus is not affected by preliminary fasting. Infectivity always increases with increasing I.F. time from a very low level, detectable after about 5 min. I.F., to a maximum somewhere between 10 and 20 hr. The number of plants infected also increases with increasing feeding time on the healthy plants, reaching a maximum after about 6 hr. of feeding. Infectivity

does not increase, but generally decreases if the aphides fast instead of feeding after cessation of I.F. The increase in infectivity with increasing test feeding time occurs whatever the I.F. time, but when the I.F. time is short few aphides are capable of causing infection at any time, and in most experiments no infections are obtained during the first hour of test feeding. Prolonging the I.F. time greatly increases the vector efficiency of the aphides, and some are able to cause infection during the first hour of feeding, though there are still more which infect later.

In most experiments which are designed to show the presence of an 'incubation period' of a virus in a vector, the I.F. times used are very short, because it is believed that long I.F. times would 'mask' the 'incubation period'. But with some of these viruses the I.F. time necessary to produce optimal infectivity of the vectors is very much longer than the 'incubation period'. The experiments therefore are made in conditions in which the vectors are only weakly infective, and the chances of their causing infection soon after cessation of I.F. are very small, or negligible. It is evident from the data published for many of these viruses that, even when the I.F. time is many times longer than the supposed 'incubation period', a very large proportion of the vectors fail to cause infection until they have fed on the healthy plants for a period corresponding to the 'incubation period'. No really satisfactory explanation has been put forward to account for this behaviour.

There is no previous record of infectivity of a virus being lost, as is beet yellows virus, more rapidly from the fasting than from the feeding vectors. Bennett & Wallace (1938) found that vectors infective with beet curly-top virus lost infectivity almost completely when they were made to fast for about 18 hr., but the loss was only temporary, and ability to cause infection was regained during four successive 5 min. feedings on healthy plants; with beet yellows virus, after prolonged post-infection fasting, the loss seems to be permanent. The performance of the starved curly-top vectors was not directly compared with that of vectors which had fed continuously for corresponding periods, but the figures given do not suggest that any infectivity was permanently lost by the fasting vectors as with beet yellows virus. Storey (1928) used fasting as a routine treatment of infective vectors of maize streak virus disease, to induce the insects to feed rapidly and uniformly; apparently this treatment did not reduce the probability of infection though again the performance of the starved vectors was not directly compared with those that had fed. No information is available about the behaviour of other aphis-transmitted persistent viruses in the fasting vectors.

The terms 'persistent' and 'non-persistent' refer to the length of time for which the viruses remain active in the vectors. In general, vectors of persistent viruses retain infectivity much longer than those of the non-persistent viruses. But the range of time over which changes in infectivity take place is very wide, especially with the persistent viruses, whose vectors may take hours, days, weeks, or even months to acquire and lose infectivity with different viruses. Non-persistent viruses all seem to be acquired and lost rapidly, but even with these there is some variation, and there can be 'overlapping' of the survival times in the vectors as

there is with best yellows and beet mosaic viruses. In these circumstances the terms 'persistent' and 'non-persistent' no longer retain their original significance.

There are other ways in which non-persistent viruses may resemble persistent ones in their behaviour. Infectivity with beet mosaic increases with increasing time on the infected plants when the aphides have not starved previously, or when the effect of preliminary fasting has disappeared. Also, in some circumstances, infectivity may increase with increasing feeding time on the healthy plants, though again this does not occur after preliminary fasting.

Thus the properties in which non-persistent viruses may resemble persistent viruses are those which are exhibited when the infectivity of the vectors is not being influenced by preliminary fasting.

It is unlikely that viruses, such as the non-persistent group, which respond to preliminary fasting of the vectors could be retained for long periods by the feeding vectors. Infectivity of previously fasted vectors decreases rapidly when they are feeding on a course of infection, and apparently the loss of infectivity is caused by the act of feeding of the vectors and not the virus source (Watson & Roberts 1939; Roberts 1940). Consequently, feeding on healthy plants, when there is no virus to replace that which is lost, must rapidly render the vectors non-infective. On the other hand, it is not necessarily true that all persistent viruses will be retained for long periods by their vectors. The length of time for which they are retained varies with the length of the I.F. time, i.e. with the virus content of the vectors. This has been shown for beet yellows virus, and also for beet curly-top virus (Freitag 1936; Bennett & Wallace 1938), and maize streak virus (Storey 1938). Plant hosts vary in the amount of virus which the vectors can obtain from them; for instance, aphides become more highly infective when fed on tobacco plants infected with potato virus *Y*, than on some varieties of potato (unpublished data), and some persistent viruses may exist in hosts which provide such a poor source of virus that the vectors would remain infective for little longer than those of the non-persistent viruses. There may also be other factors which could affect the retention of infectivity by vectors of persistent viruses, while still excluding them from the non-persistent group.

It seems, therefore, that division of the two types of viruses on the basis of the time taken for any specific reaction is not satisfactory. A more satisfactory basis, at present, would be the response to preliminary fasting. The two groups distinguished by the presence or absence of this property are probably not of equal standing; the non-persistent viruses, which possess it, appear to be a close group of aphis-transmitted viruses with similar physical properties, but the persistent viruses, which do not possess it, have many dissimilar attributes. They have vectors from different families, or even orders, of insects; their sap transmissibility varies greatly, and they respond differently to some treatments of their vectors; for instance, beet yellows and beet curly-top viruses respond differently to post-infection fasting of the vectors. There are possibly many subgroups among the persistent viruses each equivalent to the non-persistent group. Dandelion yellow

mosaic virus (Kassanis 1944), appears to be 'non-persistent', for it survives in the feeding vector for less than 1 hr., but the vectors require at least 3 hr. to become infective even when previously starved, and so the virus does not belong to the 'non-persistent' group as it is here defined.

There is some doubt as to whether such properties as those described in this paper will ever provide really satisfactory bases of classification for the insect-transmitted viruses (Bawden 1943), but the distinctions have a useful application. The variation in response to preliminary fasting and feeding time on the healthy plants can be used in experiments to separate persistent and non-persistent viruses from complexes in which they may exist; the results of such experiments will also give some information about other properties of the viruses isolated.

The general use of routine experiments, such as that described in § IV, would help to unify the results obtained by different workers, but to be of any value they should be carried out with sufficient replications to give reasonably small errors, as variation in experimental conditions causes great variation in the ease with which viruses are transmitted.

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