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* This paper had not been seen when the present paper was written; several conclusions reached agree with the results given here.

Studies on the transmission of sugar-beet yellows virus by the aphis, *Myzus persicae* (Sulz.)

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Previous studies on the relationships between plant viruses and their insect vectors have been carried out with viruses which are easily mechanically transmissible and whose vectors lose their infectivity within a few hours of removal from the source of infection. This type of virus has been called (Watson and Roberts 1939) *non-persistent*, for it was observed that the property of non-persistence of the virus in the vector was associated with other properties in which viruses of this type resemble each other, and differ from those viruses whose vectors retain their infectivity for

long periods, namely, the *persistent* viruses. It seems that these differences must lie in the nature of the viruses themselves, for viruses of both types can be transmitted by the same vector.

Sugar-beet yellows virus (Petherbridge and Stirrup 1935) seems to be a member of the *persistent* class, for its vector, *Myzus persicae*, the same insect as was used in previous work on *non-persistent* viruses (Watson 1936, 1938; Watson and Roberts 1939), remains infective for several days after removal from the source of infection (Roland 1939). Also it is not transmissible mechanically by any of the usual methods (Quanjer 1934, 1936). The present paper, therefore, describes some studies on the vector-virus relationships of this virus by the methods which have been used previously only on the *non-persistent* types.

Materials and methods

The virus used in most of these experiments was propagated from infected sugar-beet leaves kindly sent by Professor Quanjer from Wageningen, but the symptoms were indistinguishable, except in intensity, from those produced in plants to which infection was transmitted by aphides, from sugar-beet and mangold leaves collected from six different counties in England. When tested, the properties of the viruses from these different sources were also found to be identical, the differences in the intensity of symptoms being reproducible over a succession of inoculations. This suggested that the viruses collected were the same as that obtained from Wageningen, but that strains of varying virulence existed. As the symptoms produced in the glasshouses are in any case slightly different from the appearance of the disease in the field, a short description of the two main types is given.

The milder strains, which were obtained from Wageningen, Woburn (Beds), Peterborough (Lincs), Leeds (Yorks) and Tunstall (Suffolk), show their first symptoms about 10–30 days after inoculation, as small yellowish or orange-yellow patches, often at the site of penetration by the infective insects. These lesions spread, causing at first asymmetrical, and later complete, discoloration of the leaf, which eventually withers. Frequently the general withering of the leaf can be traced back to necrotic patches which appear in the yellowed areas and coalesce causing complete necrosis and death. During the winter months the leaves wither very rapidly after the yellow symptoms appear, and as they show symptoms in succession from the older leaves inwards, it frequently happens that an infected plant shows no symptoms at all, except for withered leaves remaining attached at its base. In the winter months, therefore, the more virulent

strains were used. These were obtained from Rothamsted (Herts) and Hornsea (Yorks). Their first symptoms, which in good weather appear 8–12 days after inoculation, are "vein clearing" of medium-aged leaves followed later by the appearance of local symptoms on the older leaves. The vein clearing may be very bright yellow, or have a necrotic appearance caused by collapse of the cells which lie immediately over the veins. It generally starts at the distal ends of the leaves, and may spread all over the leaf surface or remain more or less localized. In the summer months even the weaker strains produce a localized vein clearing in a few plants, but it is not so severe, or so common as with the others.

Clearing of the veins and "net-necrosis" occur as symptoms of sugar-beet yellows in the field as well as in the glasshouse, especially at Rothamsted. Even in this form it is quite easy to distinguish from the disease caused by sugar-beet mosaic virus which shows blotchy green vein-banding of all the younger leaves, whereas sugar-beet yellows symptoms never affect the very youngest leaves.

"Gummosis" of the phloem, described by Quanjer (1934) as a characteristic symptom of sugar-beet yellows, has not been found in the glasshouse plants, even in very well-established infections.

The methods of culturing and handling the insects used in the following experiments were similar to those already described for *Myzus persicae* (1936, 1938), but as the probability of infection by a single aphis per plant was found to be exceedingly low, the experiments were all carried out using five aphides for each healthy plant tested. The sugar-beet plants used for receiving the aphis transmission were at the stage when they had two leaves about 1–1½ in. long. The age of these plants varied considerably with the season, but was generally from 3 to 5 weeks. The variety used was *Kleinwanzleben E.*

The experiments were designed to test the infectivity of the aphides after varying times of feeding on infected and healthy plants. They may conveniently be divided into two groups. The first are those experiments in which each aphis group was tested for its performance on one healthy plant only, and in the second each group was tested on a succession of two or more consecutive healthy plants.

A. Infectivity of aphides tested on single healthy plants

(1) *Effect of feeding time on the infected plants*

In this experiment with the Wageningen strain of the virus, aphides were starved for a few hours, then placed on infected leaves for 5 min.

(allowing 2–3 min. actual feeding), 1 hr. or 18 hr. Aphides which were observed to be feeding were then transferred to healthy seedlings and allowed to feed for 24 hr. The experiment was repeated three times using fifteen plants for each treatment on each occasion. No infections were obtained from the 2 min. feedings. The 1 hr. feedings gave 11% and the 18 hr. feeding 69% infection. There was thus considerable increase in infectivity with increasing feeding times on the infected plants, and the chance of obtaining infective insects from very short infection feeding periods seemed to be very small.

(2) *Effect of feeding time on the healthy plants*

There were two series of tests carried out on the effect of feeding time on the healthy plant. Constant infection feeding of about 18 hr. was given in both series. In the first, using the Wageningen strain of virus, the feeding times on the healthy plants were 20, 30, 40, 90 and 180 min. In the second the Rothamsted strain was used and the times were 5, 10, 20 and 40 min.

The results are given in table 1.

TABLE 1. PERCENTAGE INFECTIVITY FOR VARYING FEEDING TIME ON HEALTHY PLANTS AFTER CONSTANT INFECTION FEEDING. W. = WAGENINGEN STRAIN, FIVE PLANTS PER TREATMENT REPEATED ON SEVEN OCCASIONS. R. = ROTHAMSTED STRAIN, FIVE PLANTS PER TREATMENT REPEATED ON FIVE OCCASIONS

	Feeding time on healthy plants (min.)						
	5	10	20	30	40	90	180
Percentage infection W.	—	—	17	23	31	58	61
Percentage infection R.	8	24	32	—	64	—	—

The infectivity of the aphides increased rapidly with increasing infection-feeding time up to about 90 min., and then more slowly. The more virulent strain used in the second series of tests gave a considerably higher level of infectivity, but the rate of increase seemed to be about the same as in the first, i.e. infectivity doubled between 20 and 40 min. It will be seen from later experiments that though increase in infectivity becomes slower for the longer feeding time it does in fact continue for several hours.

(3) *Interaction between feeding times on infected and healthy plants*

This experiment was arranged on a factorial design using five times of feeding on the infected plants and three times of feeding on the healthy plants. The results and treatments are shown in table 2.

As in the previous experiments, increasing the time spent on both infected and healthy plants increased the probability of infection, but if the data are expressed graphically, the shape of the curves suggests that infection might have been obtained for even shorter total feeding times, or else that trials over a shorter range of feeding times might demonstrate the existence of a threshold period below which the insects were incapable of completing the transmission of the virus. Such a threshold period has been described for many viruses whose other properties resemble those of sugar-beet yellows virus. This is referred to as the "latent" or "incubation" period, for with most persistent viruses it seems to be so important and prolonged that it was thought to be the time taken for a fundamental and necessary change in either vector or virus.

A second factorial experiment was carried out using shorter feeding periods on both plants. The treatments and results are given in table 3.

TABLE 2. NUMBER OF PLANTS INFECTED FOR VARYING FEEDING TIMES ON HEALTHY AND INFECTED PLANTS. FIVE PLANTS PER TREATMENT. REPEATED ON TEN OCCASIONS. WAGENINGEN STRAIN

Feeding time on healthy plants (hr.)	Feeding time on infected plants (hr.)						Mean percentage infection
	½	2	6	18	24	Total	
1	3	18	23	22	25	91	36
4	7	24	33	34	34	132	53
20	8	33	36	41	41	159	64
Total	18	75	92	97	100	382	
Mean percentage infection	12	50	61	65	66		

TABLE 3. EFFECT OF SHORT FEEDING TIMES ON INFECTED AND HEALTHY PLANTS. FIVE PLANTS PER TREATMENT; REPEATED ON FIVE OCCASIONS. ROTHAMSTED STRAIN

Feeding time on healthy plants (min.)	Feeding time on infected plants (min.)			
	7	15	30	Total
7	0	0	2	2
15	0	1	1	2
30	1	2	3	6
Total	1	3	6	10

The shortest total feeding time in which an infection was obtained was 30 min., but 7 min. on either infected or healthy plants caused infection when the other feeding was more prolonged. The results are precisely

what might be expected from those given in table 2 except that the general level of infectivity, considering the shortness of the feeding times, is higher. This, as in table 1, is due to the use of the more virulent Rothamsted strain of the virus.

There is no indication of a lower limit of time for the feeding periods, either separately or in combination, below which infectivity could not be obtained provided sufficient trials were made. As the presence of such a threshold or incubation period is so common that it is considered by most workers to be diagnostic of the type of virus for which it is found, increasing difficulty is experienced in deciding which type of insect-transmitted virus is represented by sugar-beet yellows virus. It must be concluded either that this virus is a type different from any which has yet been described, for it certainly cannot be included with the *non-persistent* viruses, or else that the possession of an incubation period is not necessarily diagnostic of the *persistent* type.

Whether sugar-beet yellows virus is in fact unique in its behaviour in relation to its insect vector remains to be proved by comparison with other *persistent* types, especially those with the same, or similar vectors, such as leaf-roll virus of potato. However, some results obtained with curly-top virus of sugar-beet, particularly by Severin (1931) and Freitag (1936), present some interesting grounds for comparison.

Severin showed (1931) that on rare occasions infections could be obtained with curly-top virus after the vector *Eutettix tenellus* had fed for only 10 min. on infected and healthy plants respectively. As curly-top has been variously reported as having an "incubation period" of "from 6 to 24 hr." and "from 1 to 44 days", Severin regarded the early infections as anomalous and suggested two possible explanations for them. One was that a few insects were capable of causing mechanical infections produced by contamination of the stylets, as transmission of the *non-persistent* viruses is commonly supposed to be effected. In support of this hypothesis he showed, by feeding non-viruliferous insects on the washings from the stylets of viruliferous insects, that these stylets were, in fact, contaminated with the virus. The other suggestion, put forward by Swezy (1930), was that a pathological condition of the oesophageal valve occasionally enabled the virus to be regurgitated directly into the plant, without its having to undergo the period of circulation through the body of the insect which they considered to take place during the normal "incubation period".

Severin adduced these arguments because he believed that the time in which the short feeding infections took place was insufficient for such a

circulation of the virus to have occurred. However, Severin's results could equally well be interpreted as showing that the infectivity of the vectors increased steadily with increasing feeding time on infected and healthy plants, in a manner similar to that exhibited by the vectors of yellows virus. In his comparative experiments the feeding times were not continued beyond a total of 4 hr., but if the early infections were caused mechanically this time should have been sufficient to give at least some indication of containing the first of two optima of infectivity, one occurring as soon after access to the source of infection as is necessary for the contamination of the stylets and delivery of the virus to the healthy plant, and one after "normal" incubation period had elasped. There is no indication of such a double optimum, and every indication that the infectivity would continue to increase in a smooth curve. Other results obtained with curly-top virus, notably those of Freitag (1936), further indicate that this increase in infectivity may continue slowly for 5 or 10 days. This suggests that the curve for increasing infectivity of the vectors of curly-top virus may be similar in shape to that of yellows virus in that, passing through zero, it rises steeply for the first part of its course and flattens out towards its maximum, but the sequence of changes is passed through more slowly than it is for yellows virus.

Thus the data published on curly-top virus may indicate that, like yellows virus, there is strictly no period after feeding on the infected plants during which the vectors are unable to transmit the virus, but merely a period of increasing infectivity towards a maximum at which all insects capable of transmitting infection will do so. The misconception seems to have arisen because when the feeding period on the infected and healthy plants is short the probability of infection is so low that it has appeared to be negligible. It seems unjustifiable to invoke complex *ad hoc* hypotheses such as those of Severin in which infections obtained after short feeding periods are assumed to be anomalous, when the results are capable of a simpler explanation which covers both short and long feeding times.

The simpler explanation suggested is that the behaviour of the virus in response to varying feeding times on both infected and healthy plants can be explained on a purely quantitative basis. This is not a new idea, for Freitag (1936), though accepting in principle the convention of a fixed incubation period, does not believe that this entails any fundamental change in either virus or vector, and Storey has on several occasions (1939) expressed the view that the behaviour of the vectors can be explained on quantitative or mechanical grounds.

To explain the present results with yellows virus, as given in tables 1

and 2, one may start by postulating that infectivity increases with increasing feeding time on the infected plants, because the amount of virus taken up by the aphides increases with the time during which virus is available for ingestion. But the infectivity of the vectors also increases with feeding time on the healthy plants, and the relative rate of increase seems to be independent of the supposed amount of virus ingested by the insect, or the time which it has taken to ingest it. The most simple case to consider is that of an insect which has fed on the infected plant only sufficiently long to give it a single infective dose of virus. This insect may deliver its infective dose immediately on penetrating the healthy plant, or within a few minutes of so doing, or the infective dose may not reach the healthy plant for some hours. Thus over a number of infection trials there is an increase in infectivity with increasing time spent on the healthy plants, which is made up of infections caused by aphides at increasingly longer intervals from the cessation of infection feeding. In other words, the effective transmission of the virus may be delayed, and, granting this hypothesis, the results show that some delay occurs in most infection trials. The cause of this delay is unknown, but at least one suggestion put forward to account for the "incubation period" may be apposite. It may be that time is required for the virus to circulate through the body of the vectors; but if so this time must vary between limits of very short and very long periods.

At first sight the observation that the increased infectivity with increasing feeding time on the healthy plant is independent of the infection feeding time is less explicable when the results for the longer feeding times are considered. These insects presumably contain several infective doses of virus, and it would be expected that the optimum infectivity should be reached more rapidly if the delay in the development of infectivity were a true threshold instead of a time lag varying from a few minutes to many hours. However, as the infective doses of virus are taken in over a period of time, which is the assumption originally made from the behaviour of the vectors in response to feeding time on the infected plants, there is no difficulty in supposing that they are given out in the same order, and if each infective dose undergoes the period of delayed transmission characteristic of the individual insect, the results of many trials among a mixed population of insects would be similar to those actually obtained.

Alternatively, the length of the delay in development of infectivity may not be an attribute of an individual aphid; any dose of virus in any insect may be ejaculated by chance early or late in the course of feeding on the healthy plant, but the doses picked up late in the infection feeding would,

on the whole, still appear later than the earlier ones. One has only to consider the case in which an infective dose picked up early in a long infection feeding might be returned later to the infected plant and thus have no opportunity of reaching the healthy plant at all. This is probably one factor which determines the slow rate of increase in infectivity with infection feeding time of 6 hr. onwards.

This explanation fits the facts so far observed for the transmission of sugar-beet yellows virus, and might apply also to curly-top of sugar-beet. They are given thus early in this paper because it is simpler to consider the behaviour of the vectors on one healthy plant only than on a succession of healthy plants, though in fact their behaviour on a succession of healthy plants seems to bear a simple relation to their behaviour on single plants. This behaviour has been explained as being due to the interaction of three factors: (1) increase in the amount of virus ingested by the aphides with increasing feeding time on the infected plants; (2) varying periods of delay in the development of infectivity at different infection trials; (3) loss of virus during and after access to the source of infection. It will be technically difficult and may be impossible to obtain direct experimental proof of the truth of these premises, though some points can be tested and are already receiving attention, but they form a convenient working hypothesis which is justified by the facts so far obtained.

B. Infections on successive healthy plants

The property of the *persistent* viruses, which has most strongly supported the view that the "incubation period" is an interval of multiplication of the virus in the body of the vector, is the capacity of the vectors for infecting a large number of plants in succession over a long period of time. We have shown (Watson and Roberts 1940) that this property is not entirely confined to the *persistent* type of virus, and that *non-persistent* viruses, which certainly do not multiply in the bodies of their vectors, can give a considerable number of consecutive infections for as long as they retain the capacity to infect. The capacity for causing consecutive infections, therefore, is not only poor evidence of multiplication of the virus, but is not even a good method of distinguishing between the two types. A more important consideration seems to be the length of time for which successive infections can be obtained, and the following experiments were designed to show the effect of varying feeding treatments on the efficiency and duration of this capacity, rather than to find the maximum number of consecutive infections which could be obtained. It is obvious that with

groups of five aphides one cannot state with certainty that all successive infections are caused by the same individual, in fact there is strong evidence to suggest that some of them were not, but as the aphides in each group were a random selection, the efficiency of the group is presumably most frequently that of its most successful individual, assuming that the infections are local and independent, and not cumulative.

(1) *Infections obtained at two successive feedings after varying infection feeding time, and with varying feeding time on the first healthy plant*

Table 4 gives the results obtained when aphides were fed for a constant period of 20 hr. on the second of two consecutive healthy plants, the times on the first plants and the infection feeding time having been varied. The results obtained on the first healthy plants have already been given in table 2, for they were not affected by the subsequent behaviour of the aphides. The present examination of the data is to determine the effect of the previous treatments on the infectivity of the vectors at their later feeding.

As on the first plants, the infectivity of the vectors on the second plants increased with increasing infection feeding time, and the increase was most rapid for the first 6 hr. Increasing time on the first healthy plant caused increased infectivity on those plants, but decreased infectivity on the second healthy plant. In fact, the longer the aphides remained on the first plants the fewer infections they were able to cause on the second, even though the time spent on the second plants was long enough for their optimum infectivity to have been exhibited.

The behaviour of the aphides in the previous experiments where they were tested on only one healthy plant for each group was explained as being due to the interaction of three factors: (1) increase in the amount of virus ingested by the aphides with increasing infection feeding time; (2) varying periods of delay in the development of infectivity in individual aphides; (3) loss of virus during and after access to the source of infection. No modification of this hypothesis is required to account for the results given in table 4.

Increasing feeding time on the infected plants caused increase in the amount of virus available for dissemination, and extension of the range of time over which it could be disseminated. The increase in the total infectivity of the aphides at both feedings is clearly seen, and it is also apparent that more insects remain infective for long periods when the infection period was prolonged. This effect is perhaps more obvious in the next experiment (table 5) where the successive feedings were carried on to a

third day, and only the aphides which had received long infection feeding retained their infectivity, although the shorter infection feeding period (4 hr.) was approaching the optimum.

TABLE 4. CONSECUTIVE INFECTIONS WITH VARYING FEEDING TIMES ON BOTH INFECTED AND HEALTHY PLANTS. FEEDING TIME ON SECOND PLANTS = 20 HR. TOTAL OF FIFTY PLANTS FOR EACH COMBINATION OF TREATMENTS

Feeding time on infected plants hr.	Infections obtained on	Feeding time on first healthy plant (hr.)			
		1	4	20	Total
½	First plant	3	7	8	18
	Second plant	1	1	1	3
	Total	4	8	9	21
2	First plant	18	24	33	75
	Second plant	21	15	2	38
	Total	39	39	35	113
6	First plant	23	33	36	92
	Second plant	30	20	9	59
	Total	53	53	45	151
18	First plant	22	34	41	97
	Second plant	36	28	9	73
	Total	58	62	50	170
24	First plant	25	34	41	100
	Second plant	32	21	8	61
	Total	57	55	49	161
Total	First plant	91	132	159	382
	Second plant	120	85	29	234
	Total	211	217	188	616

The action of the second factor, delay in the development of infectivity by the vectors, is most conspicuous when the feeding time on the first plant was only 1 hr., for then more infections were obtained at the second feeding than at the first, so that, obviously, some of the aphides could not have developed infectivity until after ceasing to feed on the first plants. Examination of the results for individual aphis groups (§ B (4)) showed that some second infections were caused by aphides which did not infect the first plant even after feeding on it for 4 hr., while a rather

larger, but still small, number of insects apparently lost their infectivity during the first feeding, so that on balance there were less infections at the second than at the first feeding. This loss is the earliest manifestation of the effect of the third factor, i.e. loss of virus from the infective aphides, whose later effect is obvious in the low efficiency of the aphides on the second day of transfer to healthy plants. The same kind of result was obtained in all the experiments on successive infections.

Loss of infectivity by the aphides is shown both by the lack of increased efficiency when the infection feeding time is increased beyond 6 hr., and in the reduction in the numbers of healthy plants infected at the later successive feedings. There is nothing in the facts so far obtained to suggest that this is not completely accounted for by virus which is returned to the infected plant during the longer infection feedings, and thus "wasted", and also by that introduced into the healthy plants, which may receive many infective doses of virus though only one is accounted for in the results. Many viruses, however, exhibit the property of gradually losing their infectivity *in vitro* in expressed sap, or even in the purified state, and it is possible that some of the loss which occurs in the insects is due to this type of inactivation.

The analysis of variance of the results, for the numbers of second plants infected, given in table 4 suggests that there is an interaction between time of feeding on infected and first healthy plants. This is probably accounted for by the smallness of the increase for increasing infection feeding time when the time spent on the first plants was 20 hr., and the fact that, when the infection feeding time was only $\frac{1}{2}$ hr. the number of second plants infected for the different feeding times on the first plants was uniformly low. The first is probably merely an indication that when the amount of virus in most of the aphides is nearing exhaustion, increased opportunity for its dissemination is not greatly effective in increasing their efficiency, and the second is really a problem concerned with the behaviour of individual aphides which is discussed in a later section (B (4)).

(2) *Consecutive infections after varying infection feeding times*

In this experiment the feeding time on the healthy plants was constant, and that on the infected plants varied. The arrangement and results can be seen in table 5.

As in the previous experiments less infection was obtained on the second day than on the first day for equal lengths of feeding time, and very much less on the third day. This does not agree with the results of Roland (1939), who concluded that the aphides were equally infective on the third day

as on the first. His results may, however, have depended upon the number of aphides used per plant, for if the number were very large, the number of plants infected might depend more on the susceptibility of the plants than the infectivity of individual aphides.

TABLE 5. INFECTIONS OBTAINED FOR 24 HR. FEEDING ON SETS OF THREE CONSECUTIVE HEALTHY PLANTS AFTER VARYING INFECTION FEEDING TIMES. FIVE PLANTS PER TREATMENT, REPEATED ON TEN OCCASIONS. TOTAL, FIFTY PLANTS FOR EACH COMBINATION OF TREATMENTS

Infection feeding time hr.	Infections obtained on		
	First plant	Second plant	Third plant
4	31	8	0
24	39	20	2
48	35	15	1
Mean percentage infection	70	28	2

Aphides with a relatively low initial charge of virus, e.g. after only 4 hr. infection feeding, appeared to be non-infective by the third day, and their infectivity on the second day was lower than that for the 24 and 48 hr. infection feeding though there was actually little difference in the total infectivity, i.e. the amount of virus, lost by the three sets of aphides.

The increase in infectivity for increasing feeding times on the infected plants did not continue after 24 hr. In fact, there was slightly less infectivity after 48 than after 24 hr. This difference is again not significant, but similar results have been observed in most of the previous experiments which have continued for more than a day, and it may be that, as Freitag (1936) found for *Eutettix tenellus*, the capacity of the aphides for causing infection diminishes with increasing age of the individuals. In a short-lived insect such as *Myzus persicae* 2 or 3 days represents a considerable period of ageing.

(3) *Successive infections on three or four plants with constant infection feeding, and varying time on the healthy plants*

The last in the series of experiments with successive healthy plants (table 6) is given in rather greater detail than the others, for the results of each repetition of the experiment are presented instead of merely the totals for all repetitions. This is not because the results are of greater importance, but because: (*a*) the treatment on the different occasions was not identical, the series being carried on to the fourth plant only on the last three

occasions; (*b*) the number of trials completed varied on the different occasions as not all the aphides completed the whole series of transfers; (*c*) the results varied considerably from occasion to occasion.

The irregular behaviour of the aphides on the different occasions may be accounted for by the fact that the experiment was carried out with the mild Wageningen strain of the virus during the winter months when development of symptoms is slow and erratic. The infectivity of the aphides was the same on the second plants as on the first, although the time spent on the first plants was 3 hr. In table 4 there was considerable reduction in infectivity after 4 hr. feeding on the first healthy plant, and there is no evidence that a large increase would result from a difference of only 1 hr.

TABLE 6. CONSECUTIVE INFECTION OBTAINED ON SETS OF THREE OR FOUR HEALTHY PLANTS, AFTER 24 HR. FEEDING ON THE INFECTED PLANTS

	Infections obtained on			
Number of plants	First plant (3 hr.)	Second plant (3 hr.)	Third plant (18 hr.)	Fourth plant (3 hr.)
12	5	4	7	—
17	6	4	13	—
15	1	1	7	—
20	13	14	15	—
Total 64	25	23	42	—
19	7	14	16	4
19	11	15	11	6
18	7	5	9	2
Total 56	25	34	36	12
Percentage infection	42	48	65	21

in the feeding times. Also from the results given in table 4 one would not expect an increase in infectivity even for 18 hr. feeding on the third healthy plant after a total of 6 hr. on the previous two. This increase is not in fact significant according to analysis of variance on the percentage infections, and it is obviously variable in occurrence and in magnitude. However, there does not appear to be reduction in infectivity such as is shown in table 4, and it might be suggested that the duration of infectivity, and possibly the period of delayed infectivity, vary with external conditions. If so, they appear to be lengthened by low temperatures or decreased daylight.

Three hours feeding on the second day (fourth plant) gave fewer infections than corresponding periods on the first day (first and second plants).

This agrees with the results obtained in both previous experiments, and the reduction seems to be about the same whether the aphid was fed for the previous 24 hr. on one, two, or three, healthy plants (compare tables 4 and 5). The mere fact of transference from plant to plant, therefore, is not able to account for decrease in infectivity, but only the time actually spent in feeding.

(4) *Distribution of infection between the successive healthy plants*

In all the experiments which were made on successive infections the performance of each individual group was recorded separately, and the results can be arranged to show the groups which infected each plant in relation to their performance on the preceding and succeeding plants. To do this the percentage of aphides which infected the later plants is calculated according to whether they did or did not infect previous plants. For example, if out of 100 pairs of successive healthy plants fifty of the first plants and twenty-five of the second plants were infected, and if it is found that twenty of these twenty-five aphis groups, or 80% of the second infections, belonged to the fifty which caused previous infection, and only five, or 20%, did not cause previous infection, then the aphis community is composed of individuals of varying efficiency, the more efficient vectors causing most of the infections on both plants. If, on the other hand, the higher percentage of successes on the second plants occurs after failure on the first, this would indicate that the general level of efficiency is uniformly low, but that a relatively high percentage of insects do not develop infectivity until some time after ceasing to feed on the infected plants. Their virus might then be available either for the first plant or for the second, but generally insufficient for both.

Table 7 gives the percentages of aphides which infected successive plants after having failed or succeeded on the previous plants. The percentages are calculated mainly from experiments whose results have already been given in tables 4–6.

With one exception the highest percentage of infections on second, third or fourth plants were obtained by aphides which had already caused infection. When the interval between the successive plants was as much as 24 hr. all the aphides which infected the final plants had given an almost complete series of consecutive infections. Thus the only most efficient vectors retained their infectivity for very long periods, and these appear to have acquired sufficient virus to infect both early and late plants. Conversely, the aphides which hold up their infectivity for very long periods are weakly infective. Although their infectivity develops late it

is less permanent than that of insects which become infective at an earlier stage.

TABLE 7. DISTRIBUTION OF INFECTIONS AMONG SUCCESSIVE HEALTHY PLANTS. DATA FROM TABLES 4–6 AND FROM ONE OTHER EXPERIMENT

Time on infected plants hr.	Performance at previous trial	Percentage giving infection at subsequent trials		
(from table 4)		First: 1 hr. Second: 20 hr.	First: 4 hr. Second: 20 hr.	First: 20 hr. Second: 20 hr.
½	+	0	0	12
	0	2	2	0
2	+	66	58	3
	0	28	4	6
6	+	69	54	26
	0	52	13	0
18	+	77	64	20
	0	68	38	12
24	+	84	53	20
	0	44	12	0
(from table 5)		First: 24 hr. Second: 24 hr.	First: 24 hr. Third: 24 hr.	Second: 24 hr. Third: 24 hr.
24	+	21	0	0
	0	12	0	0
24 and 48	+	44	4	8
	0	11	0	0
(from table 6)		First: 3 hr. Second: 3 hr.	First: 3 hr. Third: 20 hr.	Second: 3 hr. Third: 20 hr.
18	+	66	70	77
	0	37	59	52
		First: 3 hr. Fourth: 3 hr.	Second: 3 hr. Fourth: 3 hr.	Third: 20 hr. Fourth: 3 hr.
18	+	31	33	13
	0	13	5	5
(unpublished data)		First: 2 hr. Second: 22 hr.	First: 2 hr. Third: 24 hr.	Second: 22 hr. Third: 24 hr.
4	+	70	23	20
	0	62	14	12

The exception mentioned above is from table 4, and concerns the aphides which were only given ½ hr. on the infected plants. Very few of these aphides developed any infectivity and then, with one exception, after failure on the first healthy plants, indicating that the virus charge

of the aphides was only sufficient to infect the later plants if it had not been used up on the earlier ones. The aphis group which gave the single infection on a second plant after 20 hr. feeding on the first plant had also infected the first plant. This was probably caused by two aphides becoming infected in the same group, one later than the other, or by one aphis developing infectivity sufficient for two plants, just before its transfer to the second healthy plant. This single infection of a second plant appears in the table as 12% of successive infections (line 1, table 7), but this figure is obviously subject to large errors because of the small number of infections obtained on the first plants, viz. 8.

Even one second infection after 20 hr. feeding on the first plant is a higher proportion of second infections than would be expected from the results with aphides which had longer infection feeding times. It is probably accounted for by the suggestion made previously, that a larger proportion of weakly infective aphides than of highly infective ones tend to hold up their virus, i.e. there are proportionately fewer weakly infective aphides among the long infection feedings than the shorter ones.

The author has much pleasure in acknowledging the help of Miss F. M. Roberts, Ph.D., in carrying out these experiments.

Summary

The efficiency of the vector *Myzus persicae* in transmitting sugar-beet yellows virus increased greatly with increasing feeding time on the infected plants. Infection was produced on a succession of healthy plants for 1, 2, or 3 days depending on the length of the infection feeding time. The infectivity of the vectors increased with increasing feeding time on the healthy plants undergoing infection, and decreased with increasing feeding time on healthy plants prior to those on which the infection trial was made. There was no clearly defined "incubation period" of the virus in the vector, below which no insect could cause infection, but there was variation in the time between cessation of infection feeding of the aphis and the initiation of infection in the healthy plants.

The relation of this virus with its vector differ from those of the viruses already described as *non-persistent* (Watson and Roberts 1939). For the latter viruses infectivity is lost by *M. persicae* soon after cessation of infection feeding; after fasting the vectors become optimally infective almost immediately on penetrating infected tissues of the leaf. Their infectivity decreases with increasing feeding time on the infected plants,

and increases only slightly with increasing feeding time on the healthy plants.

The behaviour of sugar-beet yellows virus is compared with that of curly-top virus of sugar-beet, in which infectivity also persists for an indefinite period in the vector and increases with increasing feeding time on infected and healthy plants.

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Ocular interaction in its relation to measurements of brightness threshold

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1. Introduction

The question to which an answer is here sought is how far, if at all, the sensations from the two eyes of a subject add together in the measurement of brightness threshold. That is, do two eyes see better than one, or only equally well? Two aspects of the question have been investigated; first, the measurement of brightness threshold when the eyes are in equilibrium with a visual field of fixed brightness (steady state of adaptation); secondly, the measurement of the variation of brightness threshold with time after cutting off a conditioning field of more or less high brightness (changing state of adaptation). In an investigation of this kind the action of the eye pupils must be eliminated. This was done by the use of Maxwellian view for all visual fields, though the method of fixing the eye pupil at its