paring established varieties and new seedlings could be made reliably with 100 sets only. The glasshouse tests using aphides in standardized conditions could be done even earlier in the life of a seedling, for there would probably be ample material at the end of the second year. This method would certainly be valuable for selecting suitable parents and for rejecting very susceptible seedlings, but because of the existence of strains of viruses and the frequency with which new strains seem to occur, glasshouse tests will always need supplementing by field tests. In our work we have used only one source of potato virus *Y*, and it is possible that other sources would behave differently; with some of these the variety Katahdin may react differently and prove much easier to infect.

Our field tests show that susceptibility to infection with virus *Y* is independent of susceptibility to leaf-roll virus, for when exposed to equal chances of infection with both viruses, some varieties became mainly infected with *Y* and others with leaf roll. The reasons for these varietal differences in susceptibility are unknown, but the simplest explanation is that there is a minimal quantity of virus necessary for infection to occur and this varies with different varieties.

We wish to thank Mrs R. O. Cashen for making the statistical analysis of our results.

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Resolution of strawberry virus complexes by means of the aphis vector *Capitophorus fragariae* Theob.

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(With Plate 4 and 1 Text-figure)

Aphides (*Capitophorus fragariae*) were fed for periods of up to 24 hr. on strawberry plants infected with mild crinkle, severe crinkle or yellow-edge and then transferred to plants of the wild strawberry, *Fragaria vesca*, or of the cultivated strawberry, variety Royal Sovereign. On *F. vesca* the symptoms produced were chlorotic speckling, distortion and dwarfing of the leaves, varying in intensity, and on Royal Sovereign scattered, inconspicuous, diffuse, chlorotic spots.

The symptoms from all three sources of infection were similar and were indistinguishable from those of mild crinkle of Harris & King. The virus thus selectively transmitted is tentatively concluded to be the mild crinkle virus.

The virus was transmitted after feeding periods of 1 hr. or more and did not generally persist in the vector for more than 3 hr.

INTRODUCTION

The variation in severity of the symptoms of crinkle (Zeller & Vaughan, 1932; Ogilvie *et al.* 1934) and of yellow-edge (Harris, 1933) has given rise to the suggestion that these diseases may be caused, not by single viruses, but by mixtures or complexes of viruses or of virus strains (Zeller, 1933; Harris, 1937; King & Harris, 1942). The frequent occurrence of crinkle in association with yellow-edge or with xanthosis (which is perhaps identical with yellow-

edge) has also been noted (Zeller, 1936; Harris & King, 1942), but it is not known whether this association is obligate or fortuitous.

A method of separating the component viruses from mixtures occurring in strawberry plants would obviously help to clear up such points but, while there are several well-known methods of separating viruses from mixtures, most of them are directly applicable to sap-transmissible viruses only. Other methods depend on the fact that the viruses to be separated are transmitted by different means or possess different host ranges or that one virus is transmitted by an insect which does not transmit the other. The strawberry viruses are not sap-transmissible (Harris & King, 1940) and do not appear to be present in expressed sap (Bawden & Kleczkowski, 1945); they have few known vectors (Whitehead & Wood, 1941) and their known host range is very restricted. Such methods of separation are therefore not applicable.

A method making use of the different relationships of the potato viruses *Y* and leaf-roll to the vector *Myzus persicae* has been employed to separate these viruses (Bawden, 1943, p. 81). This method can be extended, since it has been shown that, for 'non-persistent' viruses, the efficiency of the vector is increased by preliminary fasting and short infection feeding (Watson, 1938). When aphides subjected to preliminary fasting are fed for short periods on sugar-beet infected with yellows and mosaic, they usually transmit mosaic alone when transferred to healthy plants; when long feeding periods are given, the majority of the transmissions are of yellows alone (Watson, 1945).

Preliminary experiments (begun in 1942) in the separation of the elements of strawberry virus complexes utilizing differences in vector relationships are described below.

EXPERIMENTAL

Aphides (*Capitophorus fragariae* Theob.) from a stock raised on cultivated or wild strawberry plants (which showed no symptoms and were believed to be virus-free) were transferred by means of a moistened camel-hair brush to one-half of a Petri dish. The Petri dish contained a piece of moistened filter paper so as to maintain a humid atmosphere and was tightly covered by a piece of Cellophane held in position by a rubber band. The aphides were inserted through a small hole in the Cellophane, the hole being sealed later by a moistened patch of the same material.

Aphides were allowed to fast in the Petri dish for about 18 hr. and were then transferred to detached leaves of infected plants. The leaves, whose petioles were embedded in moist sand, were from strawberry plants (var. Royal Sovereign) infected with mild crinkle, severe crinkle or yellow-edge and aphides were left on the leaves for periods of 5 min., 1 hr. and 24 hr. It was observed that aphides took about 3 min. to assume a feeding position and thus aphides left on the leaves for 5 min. probably fed for about 2 min. After being allowed to feed for these periods, two aphides were transferred to each of a number of young virus-free plants of *Fragaria vesca* and subsequently to three other series of *F. vesca* plants (Text-fig. 1). All *F. vesca* plants were kept in the glasshouse and sprayed weekly with an insecticide wash.

No symptoms appeared on any of the *F. vesca* plants receiving aphides fed for 2 min. or on any of the control plants receiving aphides direct from the stock, and only one of the sixty plants receiving aphides fed for 1 hr. became infected. Plants infected by aphides receiving a 24 hr. infection feed are indicated in Table 1.

A similar series of experiments was conducted using young strawberry plants of the cultivated hybrid variety Royal Sovereign ('Malling 40' clone) as indicators, but, owing to the faint and indefinite symptoms produced, it was not possible to diagnose infection on this variety. Royal Sovereign plants, whether infected by direct aphis transfer or by grafting to infected *F. vesca* plants, developed only very slight chlorotic spotting and sometimes showed no symptoms at all, so that infection had to be confirmed by grafting to healthy *F. vesca*.

TABLE 1. Fragaria vesca *indicators infected by aphides receiving an infection feed of 24 hr.*

Source of infection	Proportion of indicators developing symptoms* 1st transfer (10 min.) 3*A*†	2nd transfer (2 hr.) 3*B*†	3rd transfer (24 hr.) 3*C*†	4th transfer (24 hr.) 3*D*†
Yellow-edge	2/5	3/5	1/5	0/5
Severe crinkle	2/5	5/5	3/5	0/5
Mild crinkle	0/5	4/5	1/5	0/5
Total	4/15	12/15	5/15	0/15

* Numerators show number of plants infected; denominators show number of plants colonized with aphides.

† See Text-fig. 1.

Symptoms on *F. vesca* took about 20–22 days to appear and, as with Royal Sovereign, were of the same general type, no matter whether the source of infection was a plant infected with yellow-edge, severe crinkle or mild crinkle. Angular chlorotic flecks appeared on the leaves, accompanied by puckering or 'blistering' and distortion of the leaf and reduction in the size of the lamina (see Plate 4, Figs. 1, 2). Occasionally slight clearing of the leaf veins was noted as a preliminary symptom. The symptoms, however, varied in severity and it seemed that they could be classified as mild (Fig. 1) or severe (Fig. 2). Attempts to differentiate the causal viruses on

grounds other than symptomatology have not, so far, given positive results, and it may be that the variation in severity is caused by individual plant reaction or by the existence of two or more strains of one virus.

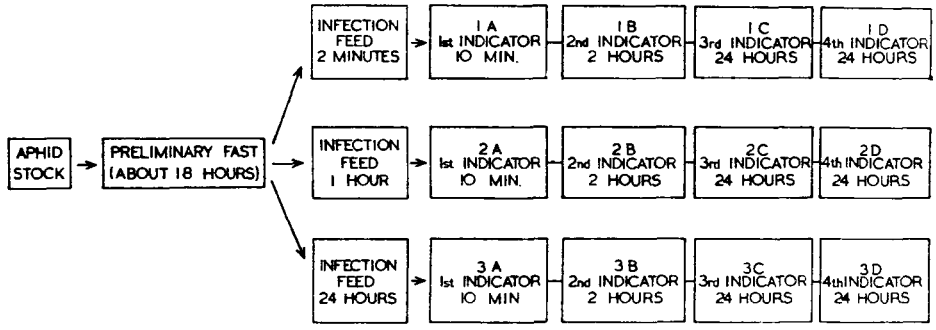
Such a variation in the severity of symptoms on *F. vesca* infected with crinkle has already been noted and the two main symptom types have been figured (Harris & King, 1942). In the present experiments, while both types occurred on plants infected from severe crinkle and yellow-edge sources, only the mild symptom type was transmitted from the plant infected with mild crinkle; in grafting experiments both symptom types have been reported from mild crinkle sources (Harris & King, 1942). It may be that the particular mild crinkle infected plant used as infector in the present experiments was infected with the mild symptom type only.

Both the mild and severe symptom types are transmitted occasionally by aphides which have fed on an infected leaf for 1 hr., but a much larger proportion of infections is produced by aphides fed for 24 hr. Combined data for the mild and severe symptom types are presented in Table 2. Each figure is a total from a number of experiments, from one to ten aphides per indicator being employed in different experiments. Larger numbers of aphides were used in experiments with short feeding times than with long feeding times, so that the increased 'efficiency' with longer feeding periods is really even greater than would appear from an examination of this table. Small-scale comparative trials suggested that fasting had no effect on the ability of the vector to transmit infection, and in most of the experiments summarized below, aphides were not subjected to preliminary fasting.

It was found that aphides which have fed on an infected leaf for 24 hr. lose their infectivity within about 3 hr. From Table 1 it will be seen that there were no infections among plants of the fourth transfer, and thus the virus does not persist in the vector for more than about 24 hr. In other experiments only one plant out of seventeen was infected by aphides which had been removed from the source of infection for 3 hr. In one case, after aphides had fed on an infected leaf for 24 hr., five of them were transferred to each of eight plants for 3 hr. and then retransferred to eight fresh plants for 21 hr. Seven of the plants of the first (3 hr.) transfer became infected and none of those of the second (21 hr.) transfer. In a second experiment, two aphides after a 24 hr. infection feed were transferred to each of ten *F. vesca* plants for 1½ hr., retransferred for ¾ hr. and again retransferred for 1 hr.; seven plants of the first series, one of the second and none of the third became infected. In a third experiment, two aphides after a 24 hr. infection feed were serially transferred for ¼, ½, ¼ and 2¼ hr. to sets of ten plants; three, five, two and two plants respectively, became infected. It would thus appear that infectivity begins to fall off within an hour and is practically nil 3 hr. after leaving the source of infection.

TABLE 2. *Effect of length of infection feed on transmission of the mild crinkle virus*

Infection feeding period	Proportion of infections	Percentage
2 min.	0/50	0
1 hr.	5/70	7
4 hr.	9/50	18
24 hr.	33/50	66
40 hr.	13/15	87



Text-fig. 1. Scheme of aphis transfers in a typical experiment.

DISCUSSION

Assuming that the mild and severe symptoms on *F. vesca* are caused by plant variation or by strains of one virus (and this explanation is supported by the similarity of the relationships of the vector to the causal viruses), it is tentatively concluded that

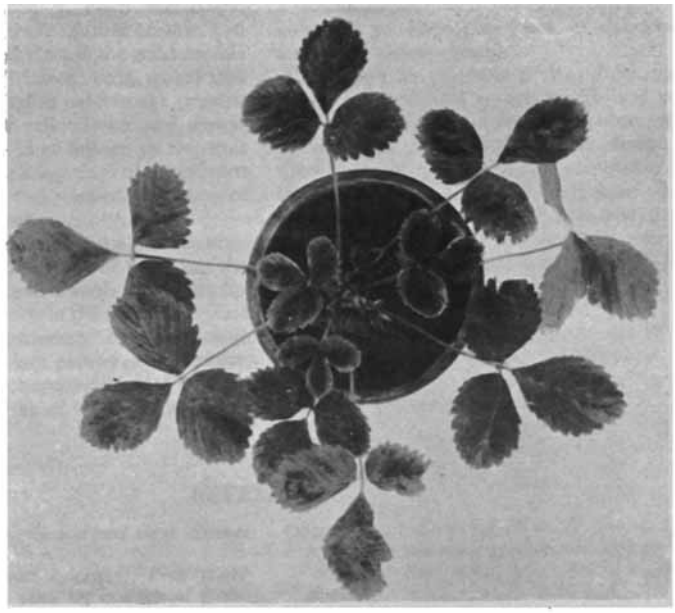


Fig. 1

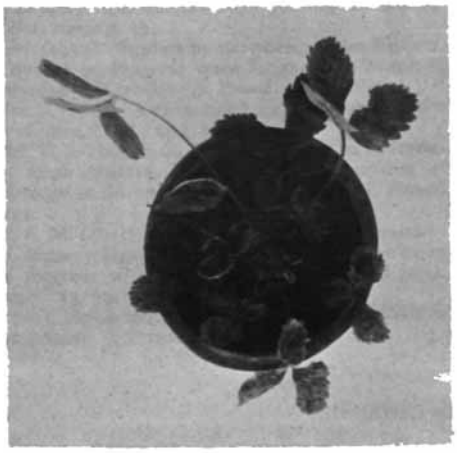


Fig. 2

Fig. 3

one virus has been isolated from plants infected with mild crinkle, severe crinkle and yellow-edge. The symptoms produced on both *F. vesca* and Royal Sovereign closely resemble those of mild crinkle, and it is thought that the isolated virus is the mild crinkle virus. It has been shown (Massee, 1935, 1942) that *Capitophorus fragariae*, the aphis used in the present experiments, is a vector of yellow-edge and severe crinkle, and it is probable that failure to transmit these symptoms is due to the conditions of the present experiments, for example, the relative shortness of the feeding periods employed.

The isolated virus (mild crinkle virus) is occasionally transmitted after infection feeds of 1 hr., but the proportion of infections increases with increasing infection feeding time. Prefasting of the aphides appears to have no effect on transmission. Thus, although the mild crinkle virus does not persist in the vector, it fails to conform to the characteristics of a 'non-persistent' virus since viruses of this type are more readily transmitted after fasting and short infection feeding (Watson & Roberts, 1939; Watson, 1938). The vector relations of the mild crinkle virus appear, however, to resemble those of dandelion yellow mosaic (Kassanis, 1944).

Although the presence of the mild crinkle virus in plants infected with severe crinkle and yellow-edge has been confirmed, it is still not clear whether it is an essential constituent of complexes producing these diseases or whether its occurrence in association with them is purely fortuitous. Isolation of other viruses from infected strawberry plants and resynthesis of severe crinkle and yellow-edge is necessary to elucidate this and work on these lines is in progress.

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EXPLANATION OF PLATE 4

Fig. 1. Infected plant of *Fragaria vesca* showing mild symptoms.
Fig. 2. Infected plant of *F. vesca* showing severe symptoms.
Fig. 3. Normal plant of *F. vesca*.

Approx. ½ natural size.

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