M. F. Day. It did not seem necessary to do this, because the virus concentration in the whole mosquito declined rapidly.

E. S. Sylvester. Does mechanically transmissible myxoma virus inoculated to mosquitoes' blood survive long?

M. F. Day. About as long as it does in serum in the laboratory.

The Specificity of Transmission of Some Non-Persistent Viruses

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ABSTRACT

When leaves infected with potato virus Y and other non-persistent viruses were exposed to ultraviolet light the infectivity of their expressed saps was reduced to about one fifth of that of the controls, suggesting that the viruses were much more concentrated in the epidermis than elsewhere in the leaves.

Non-persistent viruses are usually transmitted by aphids much more readily after about two minutes feeding on infected leaves than after longer times. With irradiated leaves there was no greater ability after short than after long infection feeding times.

Brevicoryne brassicae (L) *does not transmit cauliflower mosaic virus optimally after 2 minutes infection feeding, and its ability to transmit is not affected by irradiation of the infected leaf on which it feeds.* Myzus persicae (Sulz) *on the other hand transmits cauliflower mosaic in the same manner as other non-persistent viruses and irradiation reduces its ability to transmit after short infection feeds.*

Potato virus C is serologically related to potato virus Y and both are similarly affected by ultraviolet irradiation. But potato virus Y is readily aphid-transmissible and potato virus C, according to previous workers, not at all.

A culture of potato virus C maintained for 8 years in Nicotiana glutinosa *became transmissible by* Myzus persicae, *though less readily than potato virus Y. When inoculated to Majestic potato and returned to tobacco this culture usually again reverted to one not transmitted by aphids. The ability of a virus to be transmitted by an aphid cannot be correlated with any known physical or chemical property; nor with its distribution in the leaf or susceptibility to secretions by aphids. Present evidence suggests that it perhaps depends on the virus particle having some special group, probably only a small part of its total constitution, that combines specifically with some component of the aphid's mouthparts.*

This paper deals with viruses that resemble henbane mosaic virus in the way they are transmitted by insects. The term "non-persistent" includes them, but has also a wider connotation. They include henbane mosaic, potato Y, cucumber 1, Severe Etch, Cabbage Black ring-spot, sugar beet mosaic, and some other viruses with similar characteristics and behaviour. These are all transmissible mechanically by pricking or rubbing infected sap into healthy leaves, and their insect vectors are aphids. When vectors are starved before feeding on the infected plants they transmit most successfully after only one or two minutes feeding, and when the infection feed is prolonged their infectivity decreases to a minimum, sometimes only a tenth of their initial infectivity, after 1 or 2 hours. Unstarved aphids transmit less often and their ability to transmit does not vary with the length of infection feeding time (Watson & Roberts, 1939).

The rapidity of transmission of these viruses, the ease with which they are sap-transmissible and the lack of a latent period in the vectors point to a simple method of transmission, namely that virus particles adhere to the aphid's stylets during the infection feed, and are rubbed off into healthy tissues during the test feed, infection being caused in the same way by aphids as by pricking with a needle. However the effect of fasting, and the decline in infectivity of the vectors while feeding on infected plants suggest that transmission is affected by the physiological condition of the aphids. Also the aphids are selective in their ability to transmit; some species fail to transmit certain viruses although they can transmit others from the same host. *Myzus ornatus* (Laing), for instance, can transmit cauliflower mosaic but not Cabbage black ring-spot virus (Kvicala, 1945). The quantitative effect of fasting on the vectors' infectivity varies with different viruses and with different vectors of the same virus.

Furthermore many species of biting and sucking insects feed on the hosts of these viruses without transmitting them, and aphids feed on hosts of many other viruses which they cannot transmit. Some of the viruses that appear not to be aphid-transmissible are among the most stable and most highly concentrated in infected leaves. Tobacco mosaic

virus, for instance, is from 100 to 1000 times more concentrated in the leaf than henbane mosaic virus, and yet *Myzus persicae* if fed on a leaf containing both henbane mosaic and tobacco mosaic viruses transmits only henbane mosaic.

So far there is no satisfactory way of reconciling the apparent lack of a biological relationship between the henbane mosaic group of viruses and their vectors, with the effect of preliminary fasting of the vectors, and the relatively high degree of specificity exhibited in transmission. This is presumably because we do not know the necessary facts, or have misinterpreted them.

When aphids feed for only a few minutes on leaves their stylets penetrate no further than the epidermis (Roberts, 1940). Their high infectivity after a few minutes feeding and the later decrease could therefore be explained if most of the virus occurs in those cells. Infected leaves can be exposed to ultraviolet light in such a way that most of the virus in the epidermis is inactivated but little of that in the deeper tisues. When this is done the infectivity of sap from infected leaves is greatly reduced (Bawden, Hamlyn & Watson, 1954). Table I compares the mean number of starch lesions per half leaf (Holmes, 1931) produced by viruses coming from irradiated and unirradiated leaves.

TABLE I. Effect of Irradiation of Leaves Infected with Non-persistent Viruses, on the Numbers of Starch Lesions per Half Leaf Caused on Healthy Plants.

	Data expressed as mean log (N + 10)				
	Untreated		Irradiated		
Dilution	1/50	1/500	1/10	1/100	S.E.
Henbena mosaic virus	2.21	1.60	2.00	1.50	0.035
Cucumber virus 1	1.75	1.28	1.69	1.33	0.041
Potato virus Y	2.06	1.54	1.89	1.33	0.058
Severe Etch virus	1.94	1.44	1.92	1.46	0.176

In all, infectivity was reduced to about 1/5 of that of the controls. The volume of epidermis cannot be more than 1/5 of the whole leaf, but it apparently contains nearly 4/5 of the virus. Therefore when the aphids are feeding from epidermis they are probably tapping a much more concentrated source of virus than at other times.

When aphids are starved and then fed for short times on irradiated leaves their ability to infect becomes less than if they were fed for 24 hours on unirradiated leaves (Table II).

TABLE II. Transmission of Non-persistent Viruses from Irradiated and Unirradiated Infected Leaves. Data Expressed as Angular Transformation of Proportion of Plants Infected by Previously Starved *Myzus persicae* (Sulz), 3 Aphids per Plant.

	Untreated		Irradiated		
Infection feed	2 min.	24 hr.	2 min.	24 hr.	S.E.
Henbane mosaic virus	90	59	7	29	5.32
Cucumber virus 1	77	24	12	7	5.79
Potato virus Y	74	17	10	12	3.67
Severe Etch virus	72	20	20	7	9.35

This suggests that most of the virus acquired by vectors of the henbane mosaic group of viruses comes from epidermal cells, even that which they transmit after 24 hours on the infected plants. But there is indication of a few becoming infective by feeding on other parts of the plant, and the following results (Table III) comparing the transmission of Cabbage black ring-spot virus and cauliflower mosaic by *M. persicae* and *Brevicoryne brassicae* (L) suggest that both viruses and vectors may differ in the extent to which virus from the epidermis is transmitted. Both viruses were obtained from turnip plants, so the conditions were equivalent, but *B. brassicae* transmitted Cabbage black ring-spot optimally after 2 minutes feeding, and cauliflower mosaic after 24 hours (see also van Hoof, 1954). Moreover irradiation had little effect on the optimum infectivity of *B. brassicae* trans-

TABLE III. Transmission of Cauliflower Mosaic and Cabbage Black Ring-spot Viruses by Previously Starved *M. persicae* and *B. brassicae*. Cauliflower Mosaic Virus Data as Mean Angular Transformation of Proportion of Plants Infected by 3 Aphids per Plant. Cabbage Black Ring-spot Virus Data as Number of Local Lesions per 100 Aphids.

	Unirradiated leaves		Irradiated leaves		S.E.
Infection feeding time	2 min.	24 hr.	2 min.	24 hr.	
M. persicae					
Cauliflower mosaic virus	27	13	8	11	3.93
Cabbage black ring-spot virus	101	17	9	14	6.97
B. brassicae					
Cauliflower mosaic virus	38	64	22	52	6.01
Cabbage black ring-spot virus	30	11	6	6	3.55

mitting cauliflower mosaic, suggesting that the main source of infection was not the epidermis.

The explanation of the effect of very short feeding with most of the viruses, could be that the aphids at first pick up sap with a high concentration of virus which later pick up sap containing less. The behaviour of *B. brassicae* with cauliflower mosaic is difficult to explain on this hypothesis. The virus is obviously present in the epidermis, because *M. persicae* picks it up there, and *B. brassicae* can transmit from the epidermis because it does so with the Cabbage black ring-spot. On the evidence it seems that virus is available to aphids in deeper tissues than the epidermis though usually in too small an amount to influence transmission, but that some other factor as well as distribution affects its transmission. This factor could well be an inhibitor produced by the aphids during feeding, which might be produced by different aphids in varying quantities and at different times after starting to feed. This could account for the effect of fasting, and for some of the observed variations in the behaviour of different aphids, but it would have to be quite unprecedentedly complex and specific as an inhibitor if it is to account for everything that happens.

Besides, the inhibitor could not account for the failure of aphids to transmit tobacco mosaic virus, which is known to be highly concentrated in the epidermis, if it is also to account for the effect of fasting. The hypothesis supposes that it is not produced for the first few minutes of feeding, and tobacco mosaic virus could presumably be transmitted at that time as in henbane mosaic virus. Even if there were another inhibitor, produced continuously whether the aphid fasted or not, it would have to be a very unusual substance to inactivate tobacco mosaic virus and not affect henbane mosaic.

There are examples in the literature of insect-transmissible viruses which have lost the power to be insect-transmitted. It seemed of interest to obtain one of these and discover if any other character which might affect its insect transmissibility had altered at the same time. Potato virus C (Bawden, 1936; Cockerham, 1943), has long been recognised as a non-aphid-transmissible strain of potato virus Y, and was particularly suitable to the investigation because it has been so carefully tested by a number of workers. The first thing to test was whether its distribution in the thickness of the leaf was the same as potato virus Y. Irradiation tests using both viruses in tobacco plants, gave the local lesion counts shown in Table IV. The reduction of virus concentration by ultraviolet irradiation was the same for both viruses showing that they were similarly concentrated in the epidermis.

TABLE IV. The Effect of Ultraviolet Irradiation of Tobacco Leaves Infected with Potato Viruses Y and C on Number of Starch Lesions per ½ Leaf Caused by Inoculating Freshly Extracted Sap to Healthy Tobacco, Mean log (n+10).

	Unirradiated		Irradiated		
Potato virus Y, dilution	1/25	1/250	1/5	1/50	S.E.
	2.14	1.44	2.17	1.71	0.045*
Potato virus C, dilution	1/5	1/50	1/1	1/10	
	1.95	1.22	1.80	1.51	0.060*

*Pooled errors.

The source of potato virus C used in those experiments was an infected clone of Edgecote Purple potatoes. About 1,000 *M. persicae* were used to test its insect transmissibility in tobacco plants without success, although potato Y was repeatedly transmitted by many fewer aphids.

However another source of potato virus C (Watson, 1956) was tested and most unexpectedly this turned out to be aphid-transmissible, although the same virus culture had eight years previously been convincingly shown not to be so (Bawden & Kassanis, 1947). Between those tests in 1947 and the present ones it had been maintained by sap inoculation through a series of subcultures in *Nicotiana glutinosa* plants. These were used as a source of infection and about 1 in 20 aphids transmitted it whereas the potato Y in the same conditions 1 in 2 aphids transmitted. When the virus from *N. glutinosa*, after being transmitted by aphids to tobacco plants, was re-inoculated into Majestic or President potatoes it caused only the local necrotic lesions characteristic of potato virus C and no systemic infection. Both isolates could be taken back from the infected potato leaflets to tobacco plants, and when this was done the isolate from *N. glutinosa* was no longer aphid-transmissible; it then resembled the Edgecote Purple virus C in every respect. In several repetitions of the experiment, the virus occasionally remained aphid-transmissible after one passage through potato, and very rarely after two passages; eventually all the viruses which were passed through potato became non-aphid transmissible.

It seems almost certain that the original isolate of virus C had undergone some change that turned it into an aphid-transmissible virus during its sojourn in *N. glutinosa*. The fact that the change was reversed by passage through potato suggests that is was qualitative, and that the virus mutated to the transmissible form which infected *N. glutinosa* and the reverse mutation was induced by transfer through potato. Potato does not reduce the aphid-transmissibility of potato virus Y, so this behaviour is peculiar to the anomalous C virus.

The change could be interpreted as a quantitative one by assuming that, when virus C multiplies, some of the particles produced are always aphid-transmissible and some not, but that potato is so unfavourable an environment for the aphid-transmissible particles that only few are produced in it, whereas *N. glutinosa* is a favourable host, where they multiply up to a level easy to detect experimentally.

Whatever the actual mechanism of the change the ability of a virus to be transmitted by aphids is demonstrably a property of the virus particle, genetically determined and linked with other inherent properties. The behaviour cannot be explained as a simple effect of distribution in the leaf because both transmissible and non-transmissible strains of virus Y appear to have the same distribution. One strain and not the other might conceivably be affected by an inhibitor produced by aphids while feeding, as has been postulated, but it is unlikely that there are inhibitors of sufficient complexity and specificity to account for all the specificity which exists among aphid-transmitted viruses and between these and other viruses.

When bacteriophage invades a bacterium it first adsorbs onto the surface of the host cell. Phages which cannot invade the bacterium cannot adsorb either, but influenza virus will adsorb onto the surface of red blood corpuscles without invading. Some insect-transmitted viruses invade the tissues of their insect hosts. Others, possibly, are carried on the surfaces of cells in the blood without invading them. There is no intrinsic objection to the possibility that still others adsorb temporarily, but specifically, to surfaces in the pharyngeal area of the aphid's foregut. However Bradley & Ganong, 1955, showed that destroying the active potato Y virus at the tips of the stylets of viruliferous aphids rendered the aphids almost completely non-infective. This seems to be conclusive evidence that the virus is carried in that region and to preclude the possibility of a living surface being involved, for the stylets, so far as is known, are composed of chitin and bare of living tissue.

According to Frazer, 1944, the hemipteran-transmitted fungus, *Nematospora gossypii*, which causes internal boll disease of cotton, is carried by the vector, *Dysdercus intermedius*, in the sheaths which surround the bases of the stylets when they are retracted into the head. The fungus spores reach the stylet sheaths, and are returned to the stylet channel, by leaking between the maxillae at the point where these mouth parts come together to

enclose the anterior end of the pharynx. The maxillae are apposed in this region by muscles, which relax when the mouthparts are inserted into or withdrawn from, the leaf (Tower, 1914). There is evidence that non-persistent viruses are transmitted by aphids mainly at the time of penetration or withdrawal of the stylets, and it is conceivable that some leakage of the kind demonstrated for transmission of cotton boll disease might be involved, though it is difficult to reconcile this with the conception that only the tips of the stylets are involved in transmission. However it is almost equally difficult to reconcile the kind of specificity exhibited in the transmission of potato virus Y and potato virus C, with the simplicity of the mechanism by which they appear to be transmitted.

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