EXPERIMENTS ON THE VIRUS-VECTOR RELATIONSHIP OF MAIZE MOSAIC AND PEPPER VEIN-BANDING MOSAIC VIRUSES

AND

EXPERIMENTS ON THE TRANSMISSION OF A MOSAIC OF HIBISCUS ESCULENTUS AND OTHER MALVACEAE

BY


CONTENTS

General Introduction 1
Experiments on Maize Mosaic 7
Experiments on a Malvaceous Mosaic 20
Experiments on Pepper Vein-Banding Mosaic 27
Tables of Results 32
Summary and Conclusions 35
Acknowledgements 36
References 37
Illustrations 40
GENERAL INTRODUCTION - VIRUSES AND THEIR VECTORS

Under natural conditions, most plant viruses are transmitted by insects. Usually a specific relationship exists between the virus and the vector, any one virus being transmitted by a single vector species or by closely related species. Most vectors have sucking mouthparts and belong to the Hemiptera (aphids, mealy bugs, leaf hoppers and whiteflies). A few have biting mouthparts e.g. beetles, or rasping ones e.g. thrips.

Insect-transmitted plant viruses have been broadly divided into two classes - "persistent viruses" and "non-persistent viruses". Typically the differences between the two groups are as follows:

I. Infection feeding period

The vectors of non-persistent viruses only need to feed on an infected source plant for a few minutes to become infective. Much longer is generally required by persistent virus vectors.

II. Length of latent period and persistency

The vectors of non-persistent viruses can infect healthy plants immediately they have acquired the virus, but they soon cease to be able to do so, sometimes becoming non-infective within minutes and always within hours of leaving the infected plants. The vectors of persistent viruses cannot transfer an acquired virus immediately to healthy plants - there is a "latent period" between infection feeding and development of infectivity. Once infective, however, these vectors may remain so for long periods, sometimes for the remainder of their lives. The latent period appears to be the time taken for the virus to reach the salivary glands in sufficient concentration to give a reasonable chance of infection.
Sap-transmissible viruses are usually of the non-persistent type. Most persistent viruses are not sap-transmissible; exceptions are found among those transmitted by biting-insect vectors (Dale, 1953).

Persistent viruses are transmitted by leafhoppers, whiteflies, and thrips. Some aphid-transmitted viruses are persistent, but more are not. Most biting-insects are thought to transmit viruses by simple contamination of mouthparts, being merely occasional vectors. But for at least three viruses biting-insects are very efficient vectors, retaining the virus for some days: squash mosaic virus, transmitted by Diabrotica spp. (Freitag, 1950); Trinidad cowpea mosaic virus, transmitted by Ceratoma ruficorns (Dale, 1953); turnip yellow mosaic virus, transmitted by Phaedon cochleariae (Smith, 1951). In general characteristics these three viruses are more like some of the non-persistent mosaic group. These vectors probably transmit by regurgitation of infective juice from the fore-gut while feeding on healthy plants. Their salivary glands are vestigial and they appear to regurgitate to aid mastication. Non-persistent viruses are almost all transmitted by aphids.

There has been considerable debate as to the most suitable criterion for separating these two groups. Watson and Roberts (1939, 1940) first coined the terms "persistent" and "non-persistent", using the length of the infective period (persistency) as a basis for division.

More recently, Watson (1946) has found that the persistency of non-persistent viruses varies with:

- (a) The length of a preliminary period of fasting, before the vector is allowed to feed on an infected source plant, and
- (b) The length of the infection feeding period (period of feeding on infected source plant tissue).
She suggests that a better criterion is provided by determining the effect of a preliminary period of fasting on the vectors' ability to transmit. With non-persistent viruses, the vector's ability to transmit is increased by preliminary fasting, providing it is followed by a short (few minutes) infection feeding period. Longer infection feeding periods tend to reduce the infectivity of the vector. With persistent viruses, the chances of transmission occurring are usually proportional to the time the vectors feed continuously on an infected plant, and there is no appreciable effect from preliminary fasting.

Watson's criterion is not very satisfactory, however, because what may be called "intermediate viruses" exist. Some of these are clearly non-persistent by the earlier definition, but persistent if one accepts her more recent interpretation; these include dandelion yellow mosaic virus (Kassanis, 1947), and cacao swollen shoot virus (Posnette, 1947). These viruses fail to persist in their insect vectors, but are not transmitted when their vectors have been allowed to feed for only a few minutes on infected plants. Nor does preliminary fasting increase the efficiency of their vectors. The three beetle vectors mentioned above also transmit "intermediate viruses". For instance, Trinidad cowpea mosaic virus can persist in its vector for up to two weeks (Dale, 1953); but there is no latent period, and infectivity develops after an infection feeding period of under five minutes; nevertheless, preliminary fasting does not increase the vector's ability to transmit.

Thus, the persistency of infectivity is still thought to be the best basis for division (Bawden, 1950; Smith, 1950). No exact time can be given that will apply in all circumstances, but a convenient division is to regard viruses as non-persistent if their vectors usually cease to transmit within a day of leaving infected plants, and as persistent if their vectors normally remain infective for longer than one day. Thus, in a series of progressive 24-hour transfers of infected insects from plant to
plant, non-persistent viruses are not carried beyond the first test plant, whereas persistent viruses are usually not transmitted to the first test plant but are transmitted to some succeeding ones.

Although no absolutely hard and fast line can be drawn between the two types of virus as defined above, most viruses do fall definitely and conveniently within one or the other group. However, the question still remains as to whether these two classes do actually signify two distinct biological phenomena, or merely represent extremes in a continuous "biological spectrum", the virus-vector relationship varying with different viruses, vectors and environmental conditions. Experimental data now indicates that more than two classes are necessary, whether the divisions are made merely for convenience or to signify more fundamental differences.

The differences between the two groups, so far recognised, are almost certainly set by the viruses and not the insects, for some species, e.g., *Myzus persicae* may show both relationships with different viruses, whereas the same virus when transmitted by several insects behaves similarly with all. But this does not rule out the possibility that the insects also affect the relationship, although the macroscopic effects are not great enough to be of significance in the present classification.

In recent years, there has been considerable discussion on whether plant viruses multiply in their vectors. Such a possibility would provide a convenient explanation for some phenomena, but conflicts with others. It would not explain, for instance, why infectivity in persistent viruses often suddenly ceases while the insects are still quite healthy, not just before death. Work with sugar beet curly top (Freitag, 1936; Bennett and Wallace, 1938) and with maize streak (Storey, 1938) has shown that the infective period depends upon the length of the infection feeding period. This also is evidence, for these viruses, against the theory of virus multiplication in the vector; if the virus multiplied, one would
expect the vector to remain infective for its whole life - the period of infectivity would not suddenly cease, nor would it vary with infection feeding time.

The results of experiments testing the effect of length of infection feeding period on length of latent period are conflicting. With most viruses the latent period is found to begin from the time the vectors cease feeding on infected plants. If multiplication occurs, then the duration of the latent period would be expected to be the time required for the virus to multiply to an infective concentration. In this case it would start from the time the vectors began feeding on infected plants, and would vary with the length of the infection feeding period. But this was not found to be so with, for example, sugar beet curly top virus (Freitag, 1936) and maize streak virus (Storey, 1939). Nevertheless, in some cases, the latent period has been shown to change in duration with differing conditions. The most convincing example being demonstrated by Maramarosch (cited by Bawden, 1950). He has shown that Macropsis trimaculata that have fed for long periods on aster plants with yellows (a virus which normally has a long latent period) can infect healthy plants the day they leave infected ones.

There are three instances where convincing evidence for multiplication has been obtained. Rice dwarf virus can be transmitted by the leafhopper Nephotettix apicalis for up to seven generations (Fukushi, 1934, 1935). Similarly, clover club leaf virus can be transmitted by the leafhopper Agalliopsis novella to its progeny through the egg for at least twenty generations (Black, 1948, and work by Black cited by Bawden, 1950). An experiment by Maramarosch (1961) has given conclusive evidence for multiplication. Using the leafhopper Macrosteles divisus, he transferred the virus of aster yellows from generation to generation by macerating viruliferous insects, diluting the pulp and injecting it into healthy insects. The insects were still infective after the sixth transfer, when, if no multiplication occurred, the dilution would be about $10^{-26}$;
but aster yellows virus in insect juices has not been infective at dilutions higher than $10^{-3}$.

The best explanation for the apparently contradictory results obtained with persistent viruses is that some multiply in their insect vectors but others (probably the majority) do not. There is no evidence at all that any non-persistent virus can multiply in its vector.

The occurrence of inactive races within normally active vector species is not unknown. Storey (1932, 1933) found that active and inactive races of *Cicadulina mbila* were indistinguishable morphologically, and interbreed freely. He was able to make inactive individuals vectors by puncturing the gut just before or after infection feeding. This suggests that the difference between the races lies in the impermeability of the gut of inactive insects to the virus, preventing it from reaching the salivary glands.

As a rule, there is little difference in virus-transmitting ability between the different developmental stages, or between the two sexes. There are a few exceptions, however: for instance, it has been claimed that the thrips vectors (*Frankliniella insularis* and *Thrips tabaci*) of tomato spotted wilt virus can only acquire the virus while in the nymphal condition (Bald and Samuel, 1931).

Many workers have found that they get transmission of viruses fairly readily when large numbers of insects are used, but get few or no transmissions with single insects. This has often led to the suggestion that the insects inject sub-minimal doses of virus into the plants, the effects being cumulative, so that a plant receiving sufficient of these doses will become infected; a "mass action" effect is here implied. It seems much more likely, however, that the larger numbers merely increase the chances of infection being produced by inefficient vectors.
EXPERIMENTS ON THE VIRUS-VECTOR RELATIONSHIP OF MAIZE MOSAIC VIRUS

Introduction

In 1927, Stahl described a virus disease of corn from Cuba, naming it Stripe disease. His description and illustrations were found later (Briton-Jones, 1933) to agree closely with the symptoms of a large number of diseased plants found in Trinidad. Stahl reported that the delphacid *Peregrinus maidis* was able to transmit the disease.

Although maize mosaic, otherwise known as maize stripe, was probably present in Trinidad prior to 1929, no attention was paid to it until then. Briton-Jones (1933) started investigations on the disease, but attempts at artificial inoculation with expressed sap failed. Attempts were therefore made to transmit the disease using *Peregrinus maidis*. After a number of experiments, a limited amount of transmission was obtained, but only when the maize plants concerned were grown in poor soil.

Since 1933, there has been no other work published on this subject from Trinidad. However, Duff (unpublished) has worked on the same disease at I.C.T.A., Trinidad. In relation to the transmission of the disease in plants growing in good and poor soils, he stated that "no difficulty was experienced, the plants being grown in the normal greenhouse potting soil".

In 1941, Carter published the results of work done by him in Hawaii, on the virus-vector relationship of maize mosaic. He found the latent period to be very occasionally as short as four days, but generally between 11-29 days, a frequency graph of the recorded periods showing a normal distribution. The virus was persistent, although some of the leafhoppers lived for six days or more after the last positive transmission. The incubation period in the plant was found to range from a minimum of four days, when growth was vigorous, to a maximum of up to twenty-four days, if the growth of the plants was retarded. Carter used single
insects, giving them an infection feeding period of one day, and,
subsequently, 2-day feeding periods on each plant of a series of
healthy plants.

Object of Experiments

The original intentions were:

(a) To study the effect of the length of the infection
feeding period on the infectivity, persistency and length
of latent period. This might throw light on the subject
of multiplication of viruses in their vectors, in relation
to maize mosaic.

(b) To verify, or otherwise, Carter's results, with what is
thought to be the same virus, in Trinidad.

As the experiments progressed, unexpected results and shortage of
time and apparatus necessitated a deviation from these aims.

Symptomatology

There is considerable variation in the symptom picture
on the different varieties of maize. The following description
is of the symptoms produced by the variety Adlay in Trinidad. The
first visible symptoms were small elongated chlorotic specks near
the base of a young leaf. These specks soon increase in number,
elongate parallel to the mid-rib and more or less coalesce to form
interrupted stripes. These stripes gradually become continuous to
form an uninterrupted chlorotic band running up the leaf. Each leaf
often has more than one stripe, so that, eventually, the whole
plant takes on a chlorotic appearance. For a more comprehensive
account of the symptoms found in Trinidad, Briton-Jones' paper (1933)
should be consulted. The writer did not observe any red or
purplish-red phase, as described by Briton-Jones.

Materials and Methods

Maize seedlings were raised in 6-inch pots in a reasonably
insect-proof glasshouse. It was soon found that the soil being
used was not wholly suitable for experimentation. Nearly all the plants developed an interveinal chlorotic streaking, which was found to be due to iron deficiency, probably because of too high an alkalinity. The pH was determined by the glass electrode method to be 7.5, and there was a high free calcium carbonate content. A simple leaf-injection method (fig. 6) was used to test the leaf deficiency. Various elements were tested for, and there was a response to iron. Watering the plants with a solution of iron citrate (Fe at 5 p.p.m.) did not alter the deficiency symptoms in the leaves. In view of the fact that the deficiency would upset normal growth, and tend to mask mosaic symptoms, it was then decided to use a completely different soil. Evans (1951) potting mixture was tried and found to be quite satisfactory. This contained 2 parts of non-alkaline soil with 1 part of well-composted sawdust, fortified with ammonium sulphate, muriate of potash and superphosphate. The plants grown in this soil were used for experimental purposes when 6-18 inches high, and were of the variety Adlay.

A small percentage of the seedlings showed chlorotic symptoms. There was some infestation of *Peregrinus maidis* in the glasshouse, and mosaic disease was for a time suspected. The chlorosis was later confirmed to be of genetic origin. All the plants found to be contaminated with leafhoppers were destroyed, and the insects were killed. A number of plants were not used for experimental purposes, but left to grow in the glasshouse undisturbed. This was to ascertain whether the leafhoppers were infective and transmitting virus, which would introduce an error in the experiments. Fortunately, none of the plants developed any symptoms. Either the invading leafhoppers were not infective, or they remained permanently on one plant (which was destroyed with the leafhopper).

In the experiments, leaf cages were used (fig. 7). These were necessary to keep the leafhoppers confined on the plants, and to obviate the difficulty of catching them again when it was
necessary to transfer them to other plants. Leafhoppers in these cages were transferred to fresh healthy plants every two days during the period of the experiment. While insects were feeding on them, plants were kept in insect-proof cages (fig. 1). After transference of the insects had taken place, the plants were put back in to the glasshouse, but in a different section from the one where the unused plants were kept.

A difficulty was encountered through the fact that while feeding on the maize plants, the leafhoppers also laid eggs. These hatched in about nine days, this occurring after the plants had been returned to the glasshouse. All the plants had to be kept close together and migration from plant to plant could not be prevented. It would have been possible to mark the leaf fed upon, and to cut it off before any eggs could hatch; but this was not done as it might possibly have prevented the virus from becoming systemic. All that could be done in the circumstances was to spray the plants periodically with a suspension of chlordane. This did not prove in any way phytotoxic and was highly lethal to the vectors; but their habits reduced its efficacy, because, after attaining a certain size, they would migrate to the spaces between the stems and leaf sheaths, or to the base of the leaves, in both of which places they were protected to a large extent from the spray. Nevertheless, the number of insects in the glasshouse was kept within bounds, and, unless disturbed, the leafhoppers appeared to keep largely to one plant.

Plants used in the trials were kept under observation for up to eight weeks in the glasshouse after the insects had fed on them (unless otherwise mentioned, e.g. Experiment IV).

Colonies of Peregrinus maidis

Virus-free colonies were established during the year, but they were not used owing to the change of plan (mentioned above). It took a long time to determine definitely whether the colonies
were virus-free - from Carter's (1941) results, the symptoms might take up to fifty-three (maximum latent period and incubation period) days to develop. Two methods were used to establish colonies of this sort. One was simply to collect leafhoppers from disease-free (not necessarily virus-free) plants and put them on healthy plants in an insect-proof cage (fig.1). The other method was by collecting eggs, which are laid mainly in the mid-ribs of maize leaves; the blade was removed down to the mid-rib which was then tied to the stem of a healthy plant in an insect-proof cage. The chances that the virus is transmitted through the egg are very small. When the eggs hatch, the nymphs feed on the healthy plant, the mid-rib being dead by that time. Virus-free colonies were obtained every time either of these methods were used.

Viruliferous colonies were established by collecting the parent leafhoppers from diseased plants in the field, and introducing them into cages containing healthy plants. After a period ranging from 15-20 days the plants developed typical mosaic symptoms. Although every plant had more than ten insects feeding on it the percentage infection was not high, about 25% developing symptoms. A supply of infected plants was maintained by periodically transferring some of the leafhoppers from a diseased plant on to each of six healthy plants in insect-proof cages. The percentage transmission was again not very high: never more than 50% of the plants, and often fewer, developed symptoms (after 8-21 days). Owing to the shortage of suitably large insect cages, the last transfer of insects was made to only four plants; none of these developed symptoms, although the usual number of insects was used. It was therefore found necessary, subsequently, to use a diseased plant from one of the experiments (described later) as a host plant for the leafhoppers. The populations of insects built up from each transfer were denoted consecutively, as Series I (the population built up from the parent leafhoppers), Series II (the population built up from the first transfer), Series III (from the second transfer) etc.
When the colonies were large, they had a serious effect, through direct and indirect damage, on the plants on which they were kept. This coupled with the virus disease caused the premature death of many of the host plants.

The insects on a diseased plant were not used for experimental purposes until at least twelve days after symptoms had appeared on three leaves. Then, only adults and nymphs at least twelve days old were used. This system ensured that the insects used had been feeding on diseased tissue for at least 10-12 days.

SERIAL TRANSMISSION EXPERIMENTS

Experiment I

On 27th December, six adult insects, from the diseased host plants, were placed individually in leaf cages, these then being placed over the leaves of six healthy maize plants. The experimental procedure of transferring insects to fresh healthy plants every two days was then continued until 8th February. By this time, four of the insects had died (see Table I).

By 22nd January, no plants had developed any symptoms, and the experiment was augmented with six insects on that date, and another six on 28th January (see Table II). Like the first six insects, some of these died towards the end of the experiment. The last part of the experiment (involving insects 12-13) was discontinued on 10th March.

Results and Discussion

As shown in Tables I and II, only two plants developed symptoms in this experiment. The incubation period in one (insect No.6) was twenty-seven days, and in the other (insect No.9) twenty-five days. The latent period was twenty-four days and six days respectively.

The two plants developed symptoms in complete isolation i.e., there were no plants on either side of them in the series that also showed symptoms. This fact tends to cast doubt on the validity of these two results - the infection might have arisen...
from an outside source. If the experimental leafhoppers once became infective, one would expect them to transmit virus to more than one isolated plant. This paucity of infections was quite unexpected. Carter (1941), in Hawaii, obtained regular transmission with single insects, but he does not record the proportion of successes to failures obtained. There is no other detailed work on *Peregrinus maidis* as a vector. In Trinidad, as stated above, Briton-Jones (1933) found transmission difficult, though Duff (unpublished) says he found no difficulty. But the exact number of insects used is not given by either writer; it appears that at least several were used. In the present investigation, a much higher, though still relatively low, percentage of transmissions was obtained in the perpetuation of colonies, when larger numbers of insects were used, than in the experiments themselves. It was therefore decided to start another experiment, using several insects per test plant.

**Experiment II**

This was started on 6th February. Five insects were put in each of six leaf cages, and ten insects in six more. Apart from this, the experimental procedure was exactly the same as in the first experiment. Nymphs were used mainly, but by 10th February the majority had changed to adults. Unfortunately, the numbers of living insects soon diminished, in some instances to a considerable extent (see Table III). The experiment was continued until 10th March.

**Results and Discussion**

As shown in Table III, the percentage of plants developing symptoms was again negligible: of the four plants that did, the incubation period for two of them was eighteen days (leaf cage J, feeding time Feb. 12-14 and 14-16), and for the other two twenty-five days (leaf cages I and J, feeding time Mar. 8-10). As in the first experiment, it was by no means certain that such sporadic infections were not due to accidental infection from outside sources. However, there were three transmissions in the plants fed on by the insects
in leaf cage J, and two of these were consecutive ones in the
series. The death of many of the insects in the leaf cages
somewhat defeated the object of this experiment; but from
observations on the numbers of insects in the cages at different
dates (see Table III), it would not appear that this constitutes
the sole reason for the low proportion of transmissions obtained.
Various suggestions can be advanced to explain this; for instance,
inactive or inefficient races of the vector may exist; the
transmitting ability may vary with environmental conditions.
Carter (1941) himself found a few cases where there were breaks
in the succession of serial transmissions.

On plants of similar size to those being used in the
experiments, it was noticed that, if not confined to any particular
part of the plant, the leafhoppers generally fed right at the
base of the shoot, not far from ground level. Thus, it was thought
that the low level of experimental transmission may have resulted
from an unnatural confinement. Experiments III and IV were
conducted in an attempt to investigate such a possibility.

**Experiment III**

In this experiment, single leafhoppers were put on to
single plants, each in a separate insect cage (fig.1). Leaf cages
were not used, the insects having a free run of the plant. Not
unexpectedly, difficulty was encountered in catching and transferring
the insects, a number of which were lost in the process. Partly
due to this and partly to limited apparatus, the procedure was
discontinued after a time.

Instead of transferring the insects every two days, it
was decided to let four (only four insect cages could be spared)
insects remain on their respective plants for one month.

**Results and Discussion** (see Table IV)

Of the plants that had an insect feeding on them for two
days, only one developed symptoms.
The incubation period in this plant was twelve days. The latent period in the vector was six days (the plant was the third one that the vector fed on). Unfortunately, the vector was lost in the attempt to transfer it from this plant, so there is no record of its subsequent transmitting ability. From Table IV, it can be seen that eleven other leafhoppers were under trial, although none of these transmitted the virus. However, in all cases, the loss of the leafhopper may have occurred during its latent period - if it had been kept, the leafhopper may have developed infectivity at a later date.

Of the four plants that had single insects feeding on them for a month, again only one of the plants developed symptoms. These first appeared twenty-eight days from the time the insect was first put on it. It is impossible in this case to distinguish latent period from incubation period. Four replications is not really sufficient, and although the 25% transmission (one out of four) is similar to that obtained earlier in the establishment of colonies, it is not very significant.

However, apart from the possibility of contamination from non-experimental sources, the results provide evidence that, in Trinidad, single leafhoppers can transmit the virus of maize mosaic. But the experiment gave no indication of a significant nature whether confinement in leaf cages affected the transmitting ability of Peregrinus maidis.

Experiment IV

By the time this experiment was started, time was getting short and the reasons for the unexpected nature of the results could still only be surmised. In the time left, it was decided to carry out a further trial, using several leafhoppers per plant and growing the plants in different types of soil. As stated before, Briton-Jones (1933) found that plants grown in poor soil were more susceptible to the disease than those grown in better soil. The
writer's earlier experiments had been conducted in the light of Duff's (unpublished) claim that the disease could be transmitted in good (potting) soil. It was felt at this stage, however, that nothing connected with the transmission of this disease in Trinidad could be taken for granted. In order to test Briton-Jones' view, some poor soil was taken from some experimental plots in the I.C.T.A. grounds, on which mosaic-infected maize had been grown during the previous year. The soil there had had no recent manurial treatment and was of low fertility.

When the plants were ready for use, five growing in good (Evans potting mixture) and five in poor soil, they were put in insect-proof cages, each with five leafhoppers from infected plants on them. The insects being used in this experiment were from Series V, and they were not confined to leaf cages. Unfortunately, many of them did not take to feeding on the plants, so that some more had to be added, this time the number being made up to seven per plant in case of further losses. Observation a few days later showed there to be between four and six insects living on each plant. The insects were left on the plants, which were left undisturbed for six weeks. The experiment was started on 24th April, and the addition of insects was made on 1st May.

**Results and Discussion**

On 5th May, mosaic symptoms were noticed on two of the plants growing in poor soil. No other plants developed the disease. Although there were only five replications, these results were the most convincing the writer had yet obtained. They definitely tended to support Briton-Jones' observations that transmission was effected much more readily in plants growing poor soil. There was 40% transmission under those conditions in this experiment, whereas the transmission in the plants in good soil was nil. Time still permitted the conducting of one more experiment (V). In this it was decided to test two things: confirmation of the findings of Experiment IV, and the effect of confining the leafhoppers...
in the leaf cages. These tests had to be combined owing to a shortage of viruliferous leafhoppers. Insects from Series V failed to transmit, and so leafhoppers on the diseased plants from Experiment IV had to be used. Although breeding had occurred on these plants, the numbers of insects was not high.

**Experiment V**

On 15th May, ten insects were confined in a leaf cage to each of three plants growing in the poor soil. Three more plants were treated in the same way on 18th May, these also being in poor soil. Each leaf cage, with the insects, was transferred to fresh plants every two days during the first ten days of the experiment, after which they were left on plants for six days at a time. The length of the feeding time was increased in an attempt to test the possibility of this having an effect on transmission. After two of the six-day transfers, the experiment was discontinued.

**Results and Discussion**

None of the plants developed symptoms. Taking in to consideration the results of Experiment IV, this tends to indicate that confinement in leaf cages reduces transmission for some reason. Possibly the length of the feeding period on the plants may have an effect, but this is considered unlikely in view of the fact that the period was increased to six days, and still no transmission occurred.

**GENERAL DISCUSSION**

From previous work and the writer’s experiments, it appears that the main factors influencing the transmission of maize mosaic by *Peregrinus maidis* are number of insects per plant, soil type and confinement of the insects to particular parts of the plant.

Carter transmitted the disease successfully using single insects, in Hawaii. This does not seem to hold good for Trinidad. From the writer’s experiments, single insects may occasionally transmit the virus, but this is not absolutely certain - outside
contamination cannot be entirely ruled out under the experimental conditions prevailing (a glasshouse not completely insect-proof). When there are more than ten insects per plant, the writer found that up to 50% transmission could be obtained (work with colonies). With less than ten insects, transmission was more readily obtained when the plants were grown in soil of poor fertility. Briton-Jones could not transmit the virus using good soil (compost) and numbers (exact number not stated) of insects per plant. When he grew the plants in poor soil, one out of four plants in one experiment, and two out of many in another experiment had the disease transmitted to them. Duff states that he obtained 60% transmission using "normal greenhouse potting soil" - the number of insects per plant used is not stated, but at least more than one is indicated. One would assume the soil to be relatively fertile, but no details are given. For Trinidad, until further work is carried out on the subject, it may only be concluded tentatively that:

(a) The percentage transmission is never very high,
(b) With single insects, transmission is very occasional,
(c) Transmission increases with increase in number of insects per plant, and with decrease in fertility of soil type, these two factors probably interacting in their influence.

The writer's experiments indicate that the confinement of the vectors to a leaf cage may reduce their ability to transmit. No published work has mentioned this before, and further investigations are necessary to elucidate this subject.

Other factors may be involved in the virus-vector relationship of maize mosaic. Inactive or inefficient races of the vector may exist. Difficulty was experienced by the writer in establishing a colony of viruliferous leafhoppers - a number of batches of insects taken from highly-diseased plants failed to transmit the virus, before, finally, a successful transmission occurred. Nothing more definite can be said at the moment. It is
possible the length of the feeding time on the test plants, and the length of the infection feeding period may influence the susceptibility of the plant and the infectivity of the vector respectively. The length of the infection feeding period is known to be of importance with non-persistent viruses, but, as yet, there is no evidence to suggest that it affects persistent ones.

In Trinidad, as Carter found in Hawaii, maize mosaic virus can have a fairly long incubation period in the plant, but it varies considerably. Carter reported a range from four to twenty-four days. In the present experiments, the maximum period recorded was twenty-seven days, and the minimum eight days (as shown below, these were probably shorter periods, but these could not be accurately determined).

Barring "outside" transmissions, these experiments have shown maize mosaic virus to be persistent in Trinidad, as Carter found it (assuming it to be the same virus) to be in Hawaii. The shortest latent period was six days, compared with four days in Hawaii. However, the period may well have been shorter in some instances where it could not be determined exactly; combined latent and incubation periods of eight and eleven days were recorded - in both these cases, the latent period was probably less than six days, and the incubation period less than eight days.
EXPERIMENTS ON THE TRANSMISSION OF A MOSAIC OF HIBISCUS ESCULENTUS AND OTHER MALVACEAE

Introduction

A mosaic disease of Malvaceae was first recorded in Trinidad by Pickles and Thorold, on a Sida sp., in 1929. Since then, the number of malvaceous species known to be susceptible to mosaic symptoms in Trinidad has increased. Owen (1946) recorded the following susceptible species:

- Hibiscus esculentus L.
- Malachra alceifolia Jacq.
- Sida acuta Burm.
- S. glomerata Cav.
- S. linifolia Juss.
- S. rhombifolia L.
- S. urens L.

Owen also suggested tentatively that the mosaics of Sida spp. in Trinidad were caused by Abutilon Virus I.Baur. From grafting experiments, he considered that the agents responsible for the mosaics of H. esculentus, M. alceifolia, and certain Sida spp. should be regarded as three distinct viruses.

Mosaics of different malvaceous plants have been observed in other parts of the world: on Sida spp. throughout the Windward Islands (Owen, 1946) and in Puerto Rico (Cook, 1931); Florida and Haiti (Kunkel, 1930), Jamaica (Owen, 1946), Sierra Leone (Deighton, 1936) and Brazil (Silberschmidt, 1943); on Hibiscus esculentus in Ceylon (Fernando and Udurawana, 1942) and the Bombay district of India (Uppal et al., 1940; Capoor and Varma, 1948); on cotton in Trinidad (Dale, 1943); on Abutilon sp. in Chacachacare (personal communication); and on hollyhock in Trinidad (personal communication).
Transmission

No agent of mosaic in Malvaceae has been transmitted by inoculation of expressed sap, or by the agency of the parasite Cucurbita reflexa. There has been considerable evidence against the occurrence of seed transmission, but Keru (1933) maintained that transmission occurred to a limited extent through the seed of certain hybrids of Abutilon spp.

The insect transmission of a malvaceous mosaic was first demonstrated by Uppal et al. (1940), for the yellow vein mosaic of Hibiscus esculentus occurring in the Bombay district of India. The authors effected experimental transmission of the virus by the white fly Bemisia tabaci Genn., from H. esculentus to hollyhocks and vice-versa. Again, in Brazil, Orlando and Silberschmidt (1946) have reported transmission by B. tabaci of a mosaic virus of Sida rhombifolia. It appears highly probable that these two viruses are distinct.

Nomenclature

It was tentatively concluded by Owen (1946), from the general similarity of symptoms presented by diseased Sida spp. in Trinidad and Brazil, that the mosaics in these two countries are caused by the same virus. The same writer states that Silberschmidt's "infectious chlorosis" (Silberschmidt, 1943) was manifestly Abutilon Virus I. Baur., and so it is more than possible that this virus is also responsible for the mosaics of Sida spp. in Trinidad.

Owen (1946) gives reasons for considering yellow vein mosaic of Hibiscus esculentus being caused by a different virus. Since then, Capoor and Varma (1948) have given it a distinguishing name, viz. Ochrovena hibisciae Capoor and Varma (Hibiscus Virus I. Smith).

Object of Investigations

This was to attempt to discover the natural vector of two of the malvaceous mosaics in Trinidad. Investigations were
restricted to *Hibiscus esculentus* and *Malachra alceifolia* owing to time limited and apparatus. It was hoped that light might also be thrown on the question of whether the mosaics of these two species are, in fact, caused by different viruses. Owen's (1946) grafting experiment suggested that they are, but it is just feasible that virus might fail to pass through bud unions between certain plants.

**Symptomatology**

In both *H. esculentus* and *M. alceifolia* the mosaic consists of interveinal chlorosis, usually giving a more or less mottled appearance, except in some older leaves which may show virtually complete yellowing. The intensity of chlorosis varies considerably, tending to be most marked on the older leaves, on which yellow or even whitish patches occur. In *M. alceifolia*, but not in *H. esculentus*, chlorotic vein-banding may be present. For a fuller account of symptoms, Owen's (1946) paper should be consulted.

**Materials and Methods**

The plants were raised in a reasonably insect-proof glasshouse. They were grown from seed in 3-inch pots, and were used for experimental purposes when 3-9 inches high. In the transmission experiments, insects were collected from heavily diseased okra plants, which were readily available from peasants' plots. In the Aranguez district of Trinidad, any plot of okra plants usually showed over 95% incidence, and also a high intensity of disease. However, even high intensity does not seem to be harmful to the plant in any obvious way. The insects collected were liberated on healthy plants, one okra and one malachra, both plants being enclosed together in the insect cages illustrated (fig.1). The cages and plants were then left undisturbed for 6-8 weeks.

Observation showed that three types of insect commonly feed on okra in the Aranguez district of Trinidad:-
Aphids were usually found in colonies, but these were generally sporadic. Leafhoppers occurred in numbers up to three per leaf, and all the plants investigated were found to harbour one or more of these insects. Whiteflies were the least abundant, occurring only on some plants and in small numbers on each.

Experiments with *Aphis gossypii*

Aphids bred freely on okra, but not on malachra. On the whole, they showed a marked preference for feeding on okra, frequently deserting malachra plants and migrating to okra. When feeding in large numbers, aphids caused crinkling and inward and downward rolling of the young leaves. This had an adverse effect on the subsequent growth of the plant.

Colonies of insects on diseased leaves were collected and transferred by one of two methods to the test plants. The two methods were as follows:

(a) The insects were transferred individually by a moistened camel-hair brush, about twenty being placed on each plant. The aphids were stimulated to move before transferring, this ensuring that their probosces were not damaged when lifting them from the plant.

(b) Heavily infested parts of the diseased leaves were cut out and placed on the test plants. As the leaf
Experiments with *Empoasca gossypii*

Leafhoppers proved more difficult to catch. Special collecting apparatus was made (figs. 2 and 3), and with the help of this sufficient numbers of insects could fairly easily be collected. About twenty leafhoppers were caged on each pair of test plants. The insects gradually reduced in number, through death, over a period of about three weeks. No breeding occurred.

The experiment was repeated twice, but none of the plants developed mosaic symptoms.

Experiments with *Bemisia sp.*

These were also caught with sucking tubes (figs. 2 and 3). Muslin cages were used to confine these insects, in preference to wire-mesh ones, because of the small size of the insects.

On 9th April, ten whiteflies collected from diseased okra plants were caged with the pair of test plants. On 14th April, over twenty were collected and confined to another pair. In both instances the whiteflies disappeared rapidly, presumably through death. On inspection, after one day, only one was found feeding on an okra plant in each trial. After a few days, no insects remained alive.

In view of this, it was decided to repeat the procedure using a smaller insect cage (fig. 4), so that, the insects would have a greater chance of contacting the okra and malachra plants, before, and if, they died. In the cage, the plants were raised up on a 6-inch flower pot, so that they were near to the insects, which, when released, immediately flew to the upper part of the cage nearest the strongest light. The whiteflies were released on
28th April. On examination the day after, not one whitefly was found alive.

In an attempt to overcome the difficulty of the insects dying, a small leaf cage was devised (fig. 5) for confining the insects. With this cage a closer watch could be kept over the insects, which, while they fed, would be kept close to the leaf surface. This would ensure a greater chance of the whiteflies feeding before, and if, they died. Eight whiteflies were caged on one okra plant on 7th May. Seven survived until 10th May; on the following day only four remained alive; all were dead by 13th May, when the leaf cage was removed. Three days later the leaf on which the whiteflies had been feeding was removed to prevent any breeding taking place. The plant was subsequently kept in the glasshouse.

The okra plant used for the experiment starting on 9th April developed typical mosaic symptoms on 17th May. The malachra plant used in the same experiment did not develop any symptoms. Both these plants have since died. No other plants have produced any symptoms yet. The malachra plant of the experiment starting on 11th April has died, but all the other plants are still living.

Discussion

The symptoms on the okra plant took thirty-eight days to develop. This period is due to a possible latent period, and an incubation period in the plant. Working with H. esculentus and B. tabaci in India, Capoor and Varma (1948) found the period to vary, according to conditions, between sixteen and twenty-four days. The longer period of thirty-eight days must be due to differing conditions, mainly different plant variety and different conditions of growth.

However, at this stage one cannot draw any definite conclusions as to the vector of the mosaic of H. esculentus in
Trinidad. So far, the evidence from this experiment suggests that a *Bemisia* sp. of whitefly does transmit a mosaic of okra. The work of Capoor and Varma (1943) in India, and that of Orlando and Silberschmidt (1946) in Brazil lends support to this evidence. Taking thirty-eight days as a guide to the length of time necessary for a plant to develop symptoms (i.e. from the time the insects are first confined to the plant), one would have expected all the other plants tested to have produced symptoms by now (20th June), if they contain the virus. But this is only negative evidence; and it is only in the experiment with the leaf cage that it is certain that the whiteflies survived in sufficient numbers and lived long enough to transmit any virus they contained; and that experiment was the most recent, not being started much longer than thirty-eight days ago.

The evidence as regards Owen's (1946) contention that the agents responsible for the mosaics of okra and malachra were distinct viruses is very doubtful. But so far, if anything, it appears to be in his favour.
EXPERIMENTS ON THE VIRUS-VECTOR RELATIONSHIP OF PEPPER VEIN-BANDING MOSAIC

Introduction

A mosaic disease of sweet pepper (*Capsicum annuum* L.) was first investigated in Trinidad by Dale (1943). Subsequent to inoculation experiments, the disease was attributed to tobacco mosaic virus. Since these tests, experiments have shown that another virus much more commonly causes a mosaic in peppers, and sometimes infects tobacco. This is quite distinct from the cucumber and tobacco mosaic viruses. Dale (unpublished) has termed this new virus pepper vein-banding to distinguish it from other viruses causing mosaic of peppers.

Pepper mosaics, similar to this disease in Trinidad, have been investigated in Brazil and Puerto Rico. The one very prevalent in the Brazilian State of Sao Paulo has been described by Costa and Alves (1950). Studies on the host range and properties of the causal agent are said by these authors to indicate that it belongs to the potato virus Y group, but they put forward no evidence for this view. Cross protection experiments on the similar mosaic virus in Puerto Rico, by Roque and Adsuar (1941), suggest that this one is not related to potato virus Y. Dale (personal communication) considers that pepper vein-banding virus in Trinidad is probably related to both the Puerto Rican and Brazilian mosaic viruses. Like the Puerto Rican one, it is immunologically unrelated to cucumber or tobacco mosaic virus. The similarities between the three viruses are more striking than their differences, some of which may be more apparent than real.

Symptomatology

The symptoms in most pepper varieties consist of slight vein-clearing of the expanding leaves at the 7-10 day stage, followed by a mottling which ultimately becomes a discontinuous
and irregular dark green vein-banding. Crinkling of the leaves accompanies the mottling in many varieties. In the Large Bell Hot variety, brown vein-necrosis of the leaves appears about five days after inoculation; this is followed very quickly by abscission of the upper leaves, necrotic streaking of the stem, internally as well as externally, and finally death of the plant.

Transmission

This is effected very easily by inoculation with expressed sap, using a suitable abrasive. In 1941, Roque and Adsuar suggested that the aphid *Myzus persicae* is a natural vector of the Puerto Rican pepper mosaic mentioned above. Costa and Alves (1951) found that four aphids can transmit the pepper mosaic in Brazil. These are *Myzus persicae*, *Macrosiphum solanifoli* and two others as yet unidentified. In Trinidad, Dale (unpublished) found that *Aphis gossypii* is the chief field vector of pepper vein-banding. He also found that, in a preliminary trial, the virus "was more efficiently transmitted by vectors permitted to feed on infected plants for about five minutes only, following a period of fasting".

Object of Investigations

The preliminary vector trials carried out by Dale indicated that the virus is probably a non-persistent one. The intention was to determine if this is so, and, time permitting (these experiments had to be started late in the year), to investigate other aspects of the virus-vector relationship.

Materials and Methods

The plants were raised from seed in the glasshouse, and transplanted into 3-inch pots. A diseased plant was obtained, and, after being used as the source plant in the first insect transmission experiment, this was ground up with a pestle and mortar, and the expressed sap used to inoculate other plants, thus maintaining the supply of source plants. It was found that Large Bell Hot peppers were unsatisfactory as source plants, because of
the rapid onset of leaf abscission and death. This variety, however, provided the most convenient test plants, because of its pronounced symptoms and short incubation period. Unfortunately, a good supply of these plants was not always available. Californian Wonder peppers were not a satisfactory source plants.

Colonies of *Aphis gossypii* were raised on okra plants. All attempts to raise the aphids on pepper plants failed, for they always tended to migrate from these plants. This fact in itself tends to support the view that the virus is a non-persistent one, because only with this type would short casual feeds be adequate for efficient transmission. It was found convenient to use colonies formed on the okra plants in the experiments with okra mosaic. After the insects had been restricted to these non-susceptible plants for 6-8 weeks, it was considered that the colonies were virus-free from the pepper vein-banding virus.

The transference of aphids was done with a moistened camel hair brush. This was done carefully so as not to damage the insects' proboscies, the insects being stimulated to move before being removed.

The test plants were kept under bell jars while the insects were feeding on them. Considerable condensation occurred inside the jars and on the tips of the leaves. Many of the aphids migrated to the sides of the jars during their feeding period.

**Experiment I**

Thirty-six test plants were available for use in this experiment. It was decided to investigate not only the persistency of the virus, but also the effect of different numbers of insects on the percentage transmission. Accordingly, after a period of six hours fasting in petri dishes, the insects were allowed to feed for from two to five minutes on diseased pepper leaves. Care was taken to ensure that they fed for the requisite period of time.

In one series, single insects were then transferred to each of nine test plants, being allowed to remain on them for one
day; in another series, five insects were placed on each of a further nine plants. After a 2½-hour feeding period, the insects in both series were transferred to a second set of test plants, and twenty-four hours later they were removed and killed. The plants were then kept under observation in the glasshouse for four weeks.

Results and Discussion

None of the plants developed symptoms of the virus disease. These failures were unexpected, and the reason for them could only be surmised. Owing to force of circumstances, both the source and test plants were used at a rather late age, being about five inches or more in height. Experiment II was therefore carried out, this time using younger plants.

Experiment II

The source plant was about three inches high and only recently diseased. The test plants were in the 2½ leaf stage. The experimental procedure was the same as in Experiment I - after infection feeding, aphids were allowed to feed for twenty-four hours on each of two successive test plants, and were then killed. After a fasting period of six hours, six batches of ten insects each were treated in the above manner. Also, two batches of twenty were used - this was to test the effect of even larger numbers of aphids. To investigate the effect of longer preliminary fasting, some insects were subjected to a fasting period of twenty hours before being allowed to feed on the source plant. Four of these were treated individually in the usual manner.

Results and Discussion

Again no transmission occurred. Various suggestions can be made to account for this. The vector may be persistent, or the persistency may vary with conditions; this, however, is considered unlikely in the light of previous work by Dale (unpublished). It is possible that there are physiological races of *Aphis gossypii*, some being inactive or very inefficient vectors. Differences in host plant preferences are known - some populations take to pepper
plants much more readily than the writer's ones did. The confinement of the insects under bell jars may quite well have produced conditions unfavourable to transmission. Obviously, no definite conclusion can be drawn. Unfortunately, lack of time prevented further experiments being carried out.
**TABLE I**

<table>
<thead>
<tr>
<th>Dates of Transfer on to Successive Plants</th>
<th>Dec</th>
<th>Jan</th>
<th>Feb</th>
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<tbody>
<tr>
<td>Insects from Series I, Numbered 1 - 6.</td>
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<td>1</td>
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**Key:**

- A = Adult Leafhopper
- N = Nymph
- S = Symptoms Appeared
- D = Insect Died

**TABLE II**

<table>
<thead>
<tr>
<th>Dates of Transfer on to Successive Plants</th>
<th>Jan</th>
<th>Feb</th>
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<td>Insects from Series II, Numbered 7 - 18.</td>
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<td>Date of Transfer on</td>
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<td>Insects from Series III</td>
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<tr>
<td>Leaf Cages A - L</td>
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<td>Number of insects in cage at any date indicated by figures</td>
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<td>Initially five insects in cages A - F</td>
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<td>A - F and ten insects in cages G - L</td>
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</table>

Key: s = Symptoms Appeared.
### TABLE IV

**Insects from Series IV**

<table>
<thead>
<tr>
<th>a) Number of Transfers that Insect Underwent before being Lost.</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>b) Number of Days that Insect Lived on Healthy Plants before being Lost.</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>12</td>
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<tr>
<td>c) Number of Insects in Each of the Above Categories.</td>
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<td>3</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

**Note for (c):** Single insects were used. Some survived more transfers than others, e.g. two were lost in attempting the first transfer to the second plant, three survived only one transfer, etc. (See Text, page 14)
SUMMARY AND CONCLUSIONS

1. *Pererrinus maidis* is not a very efficient vector of maize mosaic virus in Trinidad. It may be concluded tentatively that the virus is persistent in the vector, that it has a latent period, varying between six (possibly less) days and twenty-four days, and an incubation period between eight (possibly less) and twenty-seven days. Percentage transmission appears to vary with numbers of insects per plant, fertility of soil and confinement or not to leaf cages, although the last is not at all certain.

2. *Bemisia* sp. is probably a vector of a mosaic of okra in Trinidad.

3. The virus-vector relationship of pepper vein-banding mosaic is still uncertain.
ACKNOWLEDGEMENTS

Thanks are due to Mr. W.T. Dale for his supervision of these experiments, and also to members of the Entomology Department for their help in the identification of insects.
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Figure 1.

Insect-proof cage type.

Two sizes of cage: 
(a) Height - 3 ft. 6 ins. 
   Width - 1 ft. 6 ins. sq.
(b) Height - 2 ft. 
   Width - 1 ft. sq.

Size (a) takes four 6-inch pots, and (b) one.
**Figure 2.**

Sucking tube type. (a)

rubber tubing, of any length

glass tubing, a few inches long

muslin over end of glass tubing, inside rubber tubing

Insects sucked into glass tubing, the muslin containing them.

**Figure 3.**

Sucking tube type. (b)

rubber tubing over end of glass tubing to facilitate sucking

rubber tubing of any length

muslin

specimen bottle, containing insects

Type (a) better for delicate insects such as whiteflies, as insects do not travel far and so are not sucked hard against a glass surface.
Figure 5.

Small leaf-cage type.

Lengthwise sectional view.
- glass slide
- piece of cork matting with hole (dotted lines)
- glass ring with muslin over free end
- piece of cork, showing position of hole

glass slide covering upper surface of leaf

elastic bands holding glass and cork together

piece of cork over lower surface of leaf
Small leaf-cage type.

Artificial light was directed onto upper surface of leaf to attract insects to the lower surface and encourage feeding.

Part A of diagram enlarged.

End-on sectional view of leaf cage

- retort stand supporting leaf-cage (at A)
- okra plant in pot
- leaf
- piece of cork resting on wooden cone
- glass slide
- glass ring containing insects feeding on lower surface of leaf
- hollow wooden cone held by retort stand
Leaf injection apparatus.

To test for any deficiency, a solution of the requisite element is placed in the waxed envelope. A maize leaf is cut across just below the apex and the cut end placed in the envelope as shown. The envelope is supported to prevent the leaf from breaking.
The cage's framework is covered with muslin except between the two metal crosspieces at A. The maize leaf is inserted through this opening, which is then filled with cotton wool to keep the cage insect-proof.