

## Recent Work on the Plant Viruses.

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DURING the past decade the importance of the plant viruses, both from the purely scientific and the economic points of view, has been realised and increasing attention is being paid to their study. In this article a brief account is given of some of the more recent work which, if it does not explain their nature, at least throws some light on certain aspects of the behaviour of these rather mysterious disease agents.

The field of study covered by present-day investigation of viruses is now so vast that it cannot be adequately surveyed in a single article. In order, however, to give the reader a fairly comprehensive statement of the trend of plant virus research, the subject is dealt with in three sections, and in the first of these one or two important points in the relationship of plant viruses with their insect vectors are discussed. In parenthesis it must be stated that the majority of plant viruses are dependent upon certain insects for their spread from diseased to healthy plants in the field and this relationship between insect and virus is one of considerable interest.

It has for long been assumed that the insect was more than a mere mechanical vector of the virus and evidence that supports this view has gradually been accumulating. The existence of an "incubation period" of the virus in the insect or, as it is better called, a delay in the development of infective power, the restriction of the transmitting power for a particular virus to a single insect species, the retention of the virus within the insect for long periods without recourse to a fresh source of infection, are all evidence in favour of there being some kind of obligate relationship between the insect and the virus. Some recent work by Storey<sup>1</sup> has advanced still further the knowledge of insect relationships with viruses. Working with the streak disease of maize and its insect vector, the leafhopper *Cicadulina mbila* Naude, he has shown that there exist two races of this insect, one of which, the *active* race, is able to transmit the virus, the other or *inactive* race, being unable to

do so. These two races are identical in appearance and are undoubtedly both of the same species. Furthermore by crossing the pure races Storey has demonstrated that the ability to transmit the streak virus is inherited as a simple dominant Mendelian factor linked with sex. Now comes a further step, the same worker<sup>2</sup> has shown that a simple puncture of the abdomen with a sterile needle, either following or followed by a feed on a diseased plant, sometimes caused inactive individuals of *C. mbila* to become infective. Storey concludes from these observations that in active individuals of *C. mbila* the streak virus, entering the intestine by the mouth, passes through the intestinal wall into the blood; and that, in the inactive insect, the cells of the intestinal wall resist the passage of the virus. It is recognised that there may be some secondary mechanism of resistance; nevertheless in many inactive individuals, once the barrier of the intestinal wall has been passed, the virus behaves as in an active insect. It is of great interest to find that this type of experiment has been repeated by the animal virus workers using the mosquito *Aedes aegypti* and an eastern strain of the virus of equine encephalomyelitis which this mosquito does not normally transmit. Three separate experiments were made in which the mosquitoes were allowed to feed on infected guinea pigs and half of them were then punctured in the abdomen with a small sewing needle. When the punctured mosquitoes were allowed to feed on normal guinea pigs, infection resulted, whereas the control mosquitoes that had fed on the guinea pigs at the same time but had not been punctured invariably failed to transmit the disease. Apparently this strain of the virus is incapable of penetrating the intestinal mucosa of the mosquito. If, however, it is inoculated into the body cavity by needle puncture it persists and transmission experiments are positive.<sup>3</sup>

An interesting and important point in the relationship between virus and insect is the question whether a virus can be passed

<sup>1</sup> Storey, H. H., *Proc. Roy. Soc., B*, 1932, 112, 46.

<sup>2</sup> Storey, H. H., *Proc. Roy. Soc., B*, 1933, 113, 463.

<sup>3</sup> Merrill, M. H. and TenBroeck, Carl, *J. Exp. Med.*, 1935, 62, 687-695.



from an infective parent to the progeny. This question has previously been investigated for several viruses and their insect vectors but always with negative results. In 1934, however, Fukushi<sup>4</sup> published the results of his work on the dwarf disease of rice and its insect vector, the leafhopper *Nephotettix apicalis* Motsch. var *cincticeps* Uhl. The results of these studies seem to indicate that this virus is transmitted to a certain percentage of the progeny of infective leafhoppers provided that the female of the pair is virus-infected. Progeny from crosses between uninfected females and infective males were always free from virus.

There is another and slightly different aspect of the insect relationships with plant viruses which must also be touched upon. This aspect concerns the conditions governing the movement and migration of virus-bearing insects in and about the crops. The work in question has been mainly carried out with the chief insect vector of potato virus diseases, the aphid *Myzus persicae* Sulz., and experiments have shown that humidity has a definite bearing on the movement of this insect and consequently upon the spread of the viruses. Above a temperature of 55° F. which is approximately the minimum temperature in the potato fields in the British Isles during June and July, a relative humidity of 70 per cent. and above will markedly reduce the instances of flight by *M. persicae*. At higher temperatures of 80° F. and 90° F., the effect of humidity is even more marked and flight is negligible when the humidity exceeds 85 per cent. This work has an important bearing on the selection of districts suitable for growing good "seed" potatoes. Contrary to the usual assumption, high altitudes with bleak exposed conditions are not necessarily the conditions in which aphides are scarce. Indeed the districts in which low infestations of aphides have been consistently recorded are low-lying, often almost at sea-level.<sup>5</sup>

Before leaving the subject of viruses and their insect vectors, it may be of interest to indicate some of the, as yet, unsolved problems connected with natural mode of transfer of certain viruses. Many readers of this journal will be familiar with the important virus disease of sandalwood known

as "spike" and the careful and painstaking efforts of Indian workers to ascertain the insect vector of this virus. Now it appears from a note in *Current Science*<sup>6</sup> that the number of possible vectors is being narrowed down; it has been established that the disease is insect-borne and that the vector is probably a nocturnal insect. At the moment interest is chiefly centred on three types of Pentatomidæ, two types of Jassidæ and three of Fulgoridæ. Similarly, much effort has been expended in attempting to identify the insect vector of potato virus X. This virus is exceedingly common and it undoubtedly spreads in the field; experimental evidence has been obtained of its natural spread from diseased potatoes to healthy potatoes, tobacco, tomatoes and various Solanaceous weeds. Transmission experiments with the normal insect fauna of the potato plant, carried on at Cambridge during the last four or five years, have proved negative with the exception of the work with certain species of Thrips. In all, about twelve apparently positive infections with this type of insect have been obtained in four years out of about two hundred tests. If the Thrips is the vector, therefore, and this is not yet proved, then there must be some other factor necessary for successful experimental transmission which is still to be discovered.

In the second section of this article it is proposed to discuss some of the more important advances made in the study of the virus within the plant. It is now recognised that virus diseases of plants are not necessarily due to the action of a single virus but may be caused by the concerted action of a complex of two or more viruses. As a rule the symptoms caused by two viruses are more severe than the disease due to each virus acting singly. A good example of this is the potato disease known as "crinkle" which is caused by two potato viruses of the X and Y types.<sup>7,8</sup>

Occasionally two viruses may produce a symptom picture which, while slightly different from, is no more severe than, the disease produced by either virus alone. In a case like this it would appear as if one virus

<sup>4</sup> Fukushi, T., *J. Faculty Agric. Hokkaido Imp. Univ.*, Sapporo, Japan, 1934, 37, 2.

<sup>5</sup> Davies, W. M., *Ann. Appl. Biol.*, 1935, 22, 106-115.

<sup>6</sup> Rangaswami, S., and Sreenivasaya, M., *Curr. Sci.*, 1935, 4.

<sup>7</sup> Murphy, P. A., and M'Kay, R., *Sci. Proc. R. Dubl. Soc.*, 1932, 20, 227-247.

<sup>8</sup> Smith, Kenneth M., *Proc. Roy. Soc., B*, 1931, 109, 251-267.



cancelled out the other to a slight extent. Again it is possible for a plant to be infected with two viruses but to show symptoms of only one, the second being latent and "carried" without symptoms.

The co-existence of more than one virus in the same plant leads naturally to the question of immunity and this in turn brings up the subject of virus strains. To take the latter subject first, it has been shown that certain viruses of the mosaic group may exist as a number of closely similar strains; this is particularly true of such viruses as those of tobacco mosaic, cucumber mosaic, tomato streak and potato mosaic (virus X). Large numbers of strains of the two first mentioned viruses have been isolated and some of these strains, while having the same general properties, yet may produce entirely different symptoms on similar host plants. This fact of course adds greatly to the difficulties of the identification and classification of plant viruses. Next, as regards immunity, it has been discovered that a plant which is infected with one strain of a mosaic virus is protected from further infection with another strain of the same virus, no matter how different the respective symptom pictures of the two strains may be. On the other hand several strains of the same virus will enter the same host plant if inoculated simultaneously. There is apparently no question of antibody formation in the plant involved; this non-sterile type of immunity depends entirely upon the systemic presence of the virus which entered the plant first. If this first virus is not systemic in the plant, then the second strain may enter those cells which are still free of the first strain.<sup>9,10</sup> It must be understood that this immunity is specific for like viruses and virus strains only; there is no cross-immunity conferred upon a plant against cucumber mosaic, for example, by a previous infection with tobacco mosaic. Here then, the reader will realize, is a useful means of differentiating between different viruses and virus strains and this method is particularly applicable in identifying the virus of cucumber mosaic which affects a large number of ornamental and other plants. A bright "yellow" strain of cucumber mosaic producing unmistakable symptoms can be inoculated to the plants which are suspected of

infection with the ordinary "green" strain of the virus.

The question of the origin of these different strains is an interesting one; do they arise by some mutation process during the inoculations or are they there all the time and are merely being selected out in the serial transfers of the virus? On the whole the evidence is in favour of the theory that new strains arise during inoculation studies<sup>11</sup> and some circumstantial evidence for this view is also available from a consideration of virus complexes as they occur naturally in the field. The following example will perhaps make this point clearer, potato plants are frequently found in the field affected with several strains of the mosaic virus known as X. Now it has been shown that plants infected with one strain of this virus are immune to attack by other strains,<sup>12</sup> therefore it must be assumed in such a case either that the virus X has mutated while in the plant or that these various strains were introduced simultaneously into the plant by one insect vector which seems unlikely.

The recognition that certain viruses produce necrotic spots or local lesions on the inoculated leaf has led to the development of a technique for the more accurate quantitative study of plant viruses. The use of local lesions allows the recognition of large numbers of successful transmissions on single plants and makes possible comparative estimates of virus concentrations. At high concentrations of the virus there is no direct and simple relationship between virus concentration and the number of lesions produced but it is possible within certain limits to tell which of two samples of virus is the more concentrated.<sup>13</sup> In carrying out experiments of this nature it is important to adopt a standard method of inoculation and to compare the virus samples on opposite halves of the same leaves. This is done in order to eliminate as far as possible the effects of variation in susceptibility. The kind and degree of this variation have been examined by statistical analysis and the data submitted to reduction by the analysis of variance. Plants differ greatly in their reaction to inoculation and a gradient of susceptibility was established between the different leaf positions. It was shown that

<sup>9</sup> Kunkel, L. O., *Phytopath.*, 1934, **24**, 437-466.

<sup>10</sup> Price, W. C., *Phytopath.*, 1934, **24**, 743-761.

<sup>11</sup> Jensen, J. H., *Phytopath.*, 1933, **23**, 964-974.

<sup>12</sup> Salaman, R. N., *Nature*, 1933, **131**, 468.

<sup>13</sup> Caldwell, J., *Ann. Appl. Biol.*, 1933, **20**, 109-116.



the right and left halves of a leaf responded equally to inoculation procedure.<sup>14,15</sup>

The subject of the cultivation *in vitro* of both plant and animal viruses is one which has claimed the attention of many workers. Upto the present no one has succeeded in growing a plant virus in an artificial cell-free medium and opinion seems to be divided as to whether this has been accomplished with any of the animal viruses. On the other hand several animal viruses have been grown in tissue culture or in media containing fragments of tissue and the virus of influenza is the latest addition to the animal viruses thus successfully cultivated.<sup>16</sup> For a recent review of the situation in regard to the *in vitro* cultivation of filterable viruses the reader is referred to a paper by G. Hardy Eagles.<sup>17</sup>

So far as the plant viruses are concerned, it has recently been shown that the virus of tobacco mosaic can be cultured for indefinite periods in the growing excised tips of tomato roots in a nutritive liquid. There are two rather interesting points which may be mentioned in connection with this method of virus cultivation. First it has not been found possible to inoculate the virus directly into the roots. The tomato plant itself must be inoculated and when the virus has reached the roots, the tips of these can then be cut off and cultured with the virus already within them. The second point is the absence of all symptoms in the virus-containing roots and this may be due to lack of chlorophyll in the roots since the disease symptoms characteristic of virus attack appear to be due to the destructive effect of the virus on the chlorophyll apparatus.<sup>18</sup>

Reference to the existence of viruses in the roots of plants recalls the recent discovery of a rather mysterious virus which is found in the roots of perfectly normal plants belonging to the Solanaceæ and other families. The questions of where this virus comes from and how it gets into the roots of the plants constitute some of the most interesting problems in plant virus research.<sup>19</sup>

<sup>14</sup> Samuel, G., and Bald, J. G., *Ann. Appl. Biol.*, 1933, 20, 70-99.

<sup>15</sup> Youden, W. J. and Beale, Helen Parry, *Contrib. Boyce Thomp. Instit.*, 1934, 6, 437-454.

<sup>16</sup> Wilson Smith, *Brit. J. Exp. Path.*, 1935, 16, 508-512.

<sup>17</sup> Eagles, G. Hardy, *Biol. Rev.*, 1933, 8, 335-344.

<sup>18</sup> White, P. R., *Phytopath.*, 1934, 24, 1003-1011.

<sup>19</sup> Smith, Kenneth, M., *Nature*, 1935, 136, 395-396.

The third section of this article is concerned with some aspects of the study of the virus outside the host plant. Improved methods for the ultrafiltration of viruses have been devised by Elford<sup>20</sup> who has developed a technique for the preparation of collodion membranes of uniform and graded pore size. By the passage of viruses through these membranes it is possible to calculate the particle-size of such viruses with considerable accuracy and this technique has now been applied in the measurement of the particle-size of a number of animal and plant viruses. The following are the approximate particle diameters of a few plant viruses as measured by this method; potato virus X, 80-120  $m\mu$ , tobacco necrosis virus 20-30  $m\mu$ , a new tomato virus 17-25  $m\mu$ .\*

For a proper study of the virus itself, not only from the point of view of ultrafiltration but also from other aspects, it is important that the virus suspension should be freed from as much of the extraneous matter present in plant sap as possible, and the purification of plant virus suspensions therefore forms a very important part of virus research. Stanley<sup>21</sup> working at the Rockefeller Institute in Princeton states that he has purified the virus of tobacco mosaic until he has produced a crystalline material having all the properties of that virus. Stanley inclines to the view that the virus of tobacco mosaic is an autocatalytic protein rather than a living organism.

The effect of enzymes upon viruses is interesting and important as being likely to throw light on the nature of these agents. First as regards the virus of tobacco mosaic and its reactions with trypsin; although inactivation is produced by this enzyme it is considered for the following reasons to be due partly to a virus inhibitory effect of the enzyme upon the test plant rather than upon the virus itself.<sup>22</sup> The loss of infectivity is immediate without time being necessary for the digestive action of the trypsin upon the virus. Again, the loss of infectivity is produced over a wide range of hydrogen-ion concentrations including some at which trypsin is proteolytically inactive. Lastly, the infectivity of the virus may be regained by heat, by dilution or by digestion and removal of the trypsin.

<sup>20</sup> Elford, W. J., *J. Path. and Bact.*, 1931, 34, 505.

\* 1  $m\mu$  equals one millionth of a millimetre.

<sup>21</sup> Stanley, W. M., *Science*, 1935, 81, 644-645.

<sup>22</sup> Stanley, W. M., *Phytopath.*, 1934, 24, 1055-1085.



Pepsin inactivates the virus of tobacco mosaic at pH 3 at a temperature of 37° C. and the rate of inactivation of the virus varies directly with the concentration of active pepsin. This suggests that the inactivation of the virus is due to the proteolytic action of pepsin and that the virus is therefore either a protein or very closely associated with a protein.<sup>23</sup>

The reactions of potato virus X with enzymes have also been studied.<sup>24</sup> Trypsin appears to have two distinct effects on this virus, one an immediate loss of infectivity and the other a loss of infectivity only after incubation. The fact that the immediate action of trypsin does not affect the flocculation of virus X suspensions with antiserum, whilst on incubation the serological reactions become progressively weaker, is an indication that the effects on mixing and on incubation are qualitatively different.

In the presence of pepsin as in the presence of trypsin, tobacco mosaic virus and potato virus X behave differently. As mentioned above<sup>23</sup>, 0.17 per cent. crystalline pepsin slowly inactivates tobacco mosaic virus at pH 3 and 37° C. Virus X, on the other hand, was completely inactivated by a 0.2 per cent. solution of crystalline pepsin in 3 hours at pH 4 and 38° C. Papain alone and cyanide alone had no effect on virus X but the two together inactivated it. This fact is considered to be highly suggestive that virus X contains protein.<sup>24</sup>

Finally, a new and important study, the antigenicity of plant viruses, must be briefly discussed. It has been shown by a number of investigators that antibodies reacting specifically with the sap of certain virus-infected plants can be produced by the intraperitoneal injection of rabbits with such expressed saps. The resulting *antibody*, appearing in the blood serum or body fluids of the hyperimmunized animal, reacts with the *antigen* (plant virus) in some observable way. Three types of reaction have been considered, complement fixation, precipitation and neutralization of the pathogenic properties of the virus. The following facts have now been elucidated by different workers on the serological reactions of plant viruses. The immunization of rabbits with

plant virus extracts produces sera which specifically and quantitatively neutralize the viruses concerned.<sup>25</sup> If the virus suspension be filtered through a series of graded collodion membranes the precipitin reaction is only given by that fraction of the filtrate containing the virus. In other words, "if the pores of the membrane are too fine to allow passage of the virus, then the filtrate gives no precipitin reaction. The precipitin and complement-fixation reactions are approximately proportional in strength to the quantity of virus present in the sample. These results seem sufficient to show that the antigen causing these serological reactions is the virus itself and not normal constituents of the plant sap."<sup>26</sup>

What then are the practical applications of this technique in the study of plant viruses? First, a delicate test is available for the identification of a virus since the serological reactions are specific for viruses and virus strains. Secondly, the technique can be used as a rapid and accurate means of determining the relative virus content of infective samples and further it is suggested<sup>27</sup> that the antisera afford a method for arriving at a reliable estimate of the total virus in a mixture of strains where the results of virus estimation by counting local lesions on leaves would be misleading. This applies more particularly to a mixture of two or three strains of potato virus X, some of which strains produce no local lesions on inoculated leaves.<sup>28</sup> The attempt has also been made to estimate serologically the absolute concentration of tobacco mosaic virus.<sup>29</sup>

After reading this short article some idea will have been obtained of the activity among present-day virus workers and also of the ramifications of this branch of study into the physical and chemical as well as the biological domains. The collaboration of physicists, chemists, plant pathologists and botanists in approaching this problem is to be welcomed, for only by such team work will the answer finally be obtained to the question—What is a virus?

<sup>23</sup> Stanley, W. M., *Phytopath.*, 1934, **24**, 1269-1289.

<sup>24</sup> Bawden, F. C., and Pirie, N. W. (1936, in the Press).

<sup>25</sup> Chester, K. S., *Phytopath.*, 1934, **24**, 1180-1202.

<sup>26</sup> Chester, K. S., *Phytopath.*, 1935, **25**, 702-714.

<sup>27</sup> Bawden, F. C., *Brit. J. Exp. Path.*, 1935, **16**, 435.

<sup>28</sup> Spooner, E. T. C. and Bawden, F. C., *Brit. J. Exp. Path.*, 1935, **16**, 218.

<sup>29</sup> Chester, K. S., *Science*, N. S., 1935, **82**, 17.