SOME ASPECTS OF THE PLANT VIRUS PROBLEM*

THERE appears to be rather a tendency on the part of botanists to consider the study of plant viruses a dull subject and one without any sure foundation in fact. It is hoped, therefore, in this short article to show that, on the contrary, the subject is not only an intensely interesting one, involving problems of fundamental biological importance, but is also of extreme economic importance and that plant virus workers really have a definite problem in hand.

No one at the present time knows what a virus is and this uncertainty as to its nature adds, perhaps, to the interest of the study. In speaking of a virus, stress is usually laid upon certain properties which are mainly negative in character such as inability to see the virus with the microscope, impossibility of cultivating the virus on media outside the host and the fact that viruses cannot be held back by the usual bacteria-proof filters. Improving methods of technique, however, are showing that some of these qualities are merely relative and it is already possible to photograph some viruses by means of the ultra-violet light microscope and to devise filters which will allow viruses to pass or hold them back at will according to the pore size of the filter.

In speculating upon the nature of viruses, whether of animals or plants, as a whole, it is well to remember that they are a heterogeneous collection of disease agents and it is by no means certain that they are necessarily all of the same nature. At one end of the scale is the virus of Psittacosis or parrot fever, the particle-size of which is 250 millimicrons (1 millimicron equals one-millionth of a millimitre) and which is in consequence within the range of the ordinary microscope. This virus appears to have a definite life cycle and is presumably a living organism. At the other end of the scale is the virus of foot-and-mouth disease which has a particle-size of about 10 millimicrons and is only two or three times the size of an oxyhaemoglobin molecule. It is difficult to conceive of this as a living organism. Certain plant viruses are also very small, the particle-size of tobacco necrosis virus is only 20-30 millimicrons and that of a newly described tomato virus is only 17-25 millimicrons. Again, there is the recent claim of Dr. Stanley (14) of the Rockefeller Institute in Princeton that he has succeeded in crystallising out the virus of tobacco mosaic which he considers to be an autocatalytic protein, *i.e.*, one which acts upon the cells of the host in such a way as to compel them to produce more of the same substance.

For the present it will perhaps suffice to adopt the definition of viruses given by Gardiner (5)—"as agents below or on the border-line of microscopical visibility which cause disturbance of the function of living cells and are regenerated in the process."

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In this short survey of the plant virus problem, it will only be possible to deal with one or two of the more interesting aspects of the subject and it is proposed, first of all, to discuss a few of the symptoms produced in affected plants. Since the pathological effect on the plant is almost the only criterion of the existence of a plant virus, the study of symptoms necessarily plays rather a large part. There are various kinds of virus diseases which may be loosely grouped together as follows, the *mosaic* type where attack on the chlorophyll induces the formation of mottlings or rings ; the *destructive* type which induces necroses of the cells in leaves and stems, and a third type which produces *deformities* or *overgrowth* in the affected plants.

Some of the mosaic viruses produce colour changes in the flowers of affected plants. Perhaps the best known example of this phenomenon is the so-called "Tulip-breaking" in which tulips affected with a mosaic virus produce variegated flowers. Certain of these tulips with variegated flowers at one time fetched large sums of money owing to the mistaken idea that they were new varieties, whereas they were in reality only diseased specimens of self-coloured varieties. References to this tulip "breaking" may be found in the literature of very early times. Thus, the first record is a description published in 1576, and other accounts of this variegation in tulips appeared in 1622 and 1670. It was in this latter account that the suggestion was first made that the variegated tulip might be diseased. In the Rembrandt Exhibition recently held in Amsterdam were paintings of tulips by Dutch artists of the sixteenth and seventeenth centuries, and many of these tulips showed a typical mosaic infection. Just recently, growers of the favourite blood-red variety of wallflower have been perturbed by the appearance of an ugly yellow stripe or flecking in the red flowers and this has led to many complaints from customers that their colour schemes have been spoiled ; similarly with self-coloured stock (10). The variegation in these flowers has been shown to be due to a virus carried to the plants by a species of greenfly from virus-infected broccoli of cauliflowers in the neighbourhood.

In the writer's opinion viruses play a larger part in the production of variegations in flower colours than is usually supposed. For instance, inoculations from the petals of common variegated mauve and white and mauve and yellow violas, picked at random from the garden, to healthy tobacco plants of the White Burley variety, produced in those plants a virulent mosaic disease. The virus is also capable of infecting several other species of Solanaceous plant. Experiment seems to show that the virus causing this variegation is a strain of cucumber mosaic virus (cucumber virus 1).

Some of the mosaic viruses affecting ornamental plants may produce little effect on the plant other than the change in the colour of the flowers. It is quite likely therefore that a systematic enquiry into the question would show that other familiar flower variegations may be due in part to virus infection. There seems, however, to be a common element in the appearance of this type of variegation, *i.e.*, a pencilling or flecking of the colours and a break in the hard line dividing two colours.

The next question is the important one of how plant viruses are transmitted in nature from diseased to healthy plants. The majority of plant viruses depend upon insects for their dissemination from plant to plant and this relationship between insect and virus is one of considerable interest. The insects concerned in the spread of plant viruses are nearly all of one type, a type of insect which feeds in a particular way which seems to be well adopted for the injection of the virus into the plant. These insects belong to the order Hemiptera and are of the sap-sucking type.

Insects are not merely mechanical vectors of the virus but in all probability some kind of obligate relationship exists between the two. The following facts seem to bear this out—certain viruses cannot be transmitted from diseased to healthy plants except by the agency of insect and often only by one species of insect or one type of insect and not by other closely related species ; some insect vectors having fed once upon a virus-diseased plant remain infective for the rest of their lives without the necessity for further recourse to a source of virus infection. This suggests that the virus actually multiplies in the body of the insect. Further, some insects do not become infective until a minimum time has elapsed after feeding upon a virus-infected plant. This is often referred to, perhaps on insufficient grounds, as the "incubation period" of the virus in the insect. A better term would be " a delay in the development of infective power within the insect". This day may be as long as ten days in some cases.

It is not possible to deal at length with the question of the insect relationships of plant viruses, but space permits touching upon some recent interesting work on this subject. Storey (15), working upon the leaf-hopper which transmits the streak disease of maize in East Africa, has found that there exist two distinct races of this insect. One race which can transmit the virus and one race which is unable to do so ; these races are termed *active* and *inactive* respectively. There is no visible difference between the inactive and active races and both are of the same species. Further, Storey has shown that if a puncture is made with a fine needle in the wall of the gut or alimentary canal of the inactive insect, the insect then becomes capable of transmitting the virus. It would appear from this that there may exist some factor or factors connected with the structure of the wall of the alimentary canal in inactive insects which prevents the virus from passing through into the blood and so reaching the salivary glands whence it is injected into the plant.

The next point concerns the mechanism of movement of the virus in the plant. Since most viruses rapidly become systemic in their hosts, there is evidently an efficient means of transport about the plant. It has been shown by Bennett (1), Caldwell (2) and others that if the phloem in a portion of the stem of a plant is destroyed by steaming, the virus cannot pass over this bridge of dead tissue. In other words the virus is moving in the phloem, but not in the xylem. The disease will develop normally in whichever half of the plant is inoculated, but the virus will not pass from the upper to the lower nor from the lower to the upper half, across the bridge of dead tissue.

The general movement of a virus about the infected plant has been well demonstrated by Samuel (9). His experiments show that there is no movement of tobacco mosaic virus from the inoculated leaf for a period of 3-4 days. The virus then passes out of the inoculated leaf and travels rapidly to the roots of the plant ; about a day later it travels with equal rapidity to the top of the plant. In pot plants the more mature leaves become successively invaded from the top downwards and from the bottom upwards until the plant is completely invaded by the virus.

The movement of the virus in the plant thus seems to be of two kinds : first, a very slow cell-to-cell movement *via* the connecting protoplasmic bridges until the phloem stream is reached, when the main and most rapid movement about the plant begins. Further confirmation of this is afforded by some experiments with a newly discovered virus known as tobacco necrosis (13). This virus produces only necrotic symptoms and thus etches out, as it were, its own movement through the plant. Photographs have been taken at two-day intervals of the path followed by the virus in the leaves of cowpea (*Vigna sinensis*). The first six photographs show merely a gradual increase in size of the lesion at the point where the virus has entered the leaf. As soon, however, as the virus enters the phloem it begins to travel rapidly through the leaf, moving in 48 hours over a much greater distance than in the whole of the preceding 12 days' slow cell-to-cell movement.

On another aspect of the subject two interesting discoveries have recently been made : firstly, it has been found that some plant viruses exist in a number of closely allied strains, and secondly, it has been shown that infection with one strain of a virus will immunise a plant from infection with another strain of that virus. Space will not suffice to allow of a discussion as to whether these strains actually arise by mutation from existing strains, but the evidence rather indicates that this is the case.

The immunity conferred upon a plant by a virus strain against other strains of the same virus is of the non-sterile type. There is apparently no question of the production of antibodies and it is the presence of the first virus which inhibits the entrance of the second strain. This type of immunity is well shown in the case of potato virus X (8), tobacco (6) and cucumber mosaic viruses (7) and by the virus of tomato streak (11). All these viruses exist in strains and the "green" and "yellow" strains of the tobacco or cucumber viruses are particularly suitable for this kind of experiment. If a healthy tobacco plant and one systemically infected with a "green" strain of tobacco mosaic are inoculated with a "yellow" strain, the healthy plant develops the yellow spots characteristic of this virus, while the plant already infected with the "green" strain is protected against invasion by the"yellow" strain. A similar protective action is exerted in the case of a plant infected with a "yellow" strain against invasion by the "green" strain. It should perhaps be emphasised that the presence of one virus in a plant is no bar to the entrance of a second virus of a different type, the cross immunity holds good only for like viruses and virus strains. This kind of immunity therefore is likely to prove a useful tool in the work of classifying viruses and in distinguishing like from unlike viruses in those cases where diagnosis by symptoms alone is unreliable.

A possible practical application of this type of immunity lies in the protection of a crop from infection with a severe virus by previous artificial infection with a mild strain of the same virus. Here, however, lie a number of pitfalls, chief of which is the unfortunate liability of certain viruses, even when in a mild form, to give rise jointly with another virus of a different type, to a much more severe disease than is produced by either virus acting separately.

Mention must be made of a comparatively new method of approach to the plant virus problem, *i.e.*, the discovery that the introperitoneal injection of rabbits with plant virus extracts induces the production of *antibodies* in these animals. These antibodies react specifically with the *antigen* (virus sap) in some observable way. Three types of immunologic reactions have been demonstrated, complement-fixation, precipitation and neutralisation of the pathogenic properties of the virus. Such neutralisation is specific for each virus, thus, tobacco mosaic virus is inactivated only by anti-tobacco mosaic serum, and tobacco ringspot virus only by anti-tobacco ringspot serum, and so on. The cross specificity is absolute and the addition to any of the viruses of a heterologous antiserum exhibits no effect. This specificity, however, does not extend to distinctions between virus strains even when the strains produce very different symptoms in ths host plants. (Chester (3)).

This new technique is likely, therefore, to prove a useful tool in the difficult task of classifying and differentiating plant viruses.

Since viruses are so often spoken of as filter-passing or ultra-microscopic, and described by other adjectives referring to their small size, it may be of interest to give a few details of the actual magnitude of some viruses. The sizes of virus particles can be measured with fair accuracy by means of ultra-filtration through collodion membranes, the pore size of which can be measured. These membranes are prepared by a special technique devised by Dr. Elford (4) of the National Institute of Medical Research at Hampstead and the process of their manufacture is too complicated to describe here. It has been found by the application of this technique that plant viruses vary very much in their particle size, ranging from 75 to 100 millimicrons for a potato virus down to 17-25 millimicrons for a new tomato virus.

In conclusion, it is proposed to give a short account of an interesting new virus, because it well illustrates the kind of problem with which the virus worker is sometimes faced. It has been found at Cambridge (12) that a high proportion of the normal stock of healthy tobacco plants carry a virus in the roots but not in the stem and show no signs of disease during the whole of their life. Under certain conditions, however, in the winter and early spring the virus may pass up into the plant and develop disease symptoms in the lower leaves. Unlike most other plant viruses, this virus does not become systemic in the host. Further, and this is the most interesting point, tobacco seedlings which by available methods of inoculation have been shown to be virus-free, yet contain the virus in their roots in quite large quantities some five weeks later. The following experiment illustrates this. Seed from a White Burley tobacco plant grown in the insect-proof house was sown in sterilised sand in a "cellophane" cage in the glass-house. From the resulting seedlings a number of small plants were chosen and all the roots cut off except that one root was left on each plant. The roots of each plant thus removed, were ground up and the resulting paste inoculated separately to three or four cowpeas, a plant which is extremely sensitive to the virus. The tobacco plants were then repotted in sterilised soil and allowed to grow on ; from this number 48 plants, the roots of which had given no reaction upon the cowpeas, were selected for a second test. This was made, again to cowpeas, 5 weeks after the first test. The plants were by this time about 8 inches across with a well-developed root system, and showed no unusual symptoms. Of these 48 plants 32 gave a virus reaction. In considering these results certain other facts must be borne in mind ; exhaustive tests make the possibility of outside infection by seed, soil or water-transmission unlikely, though seed transmission in some form cannot definitely be excluded. The virus is not insect borne.

There seem to be three possible explanations of this problem : first, it may be assumed that the virus is present all the time in the stem, but present either in a non-virulent form which requires to gain virulence by concentration in particular cells of the root, or else in a dilution too great to give a positive reaction on inoculation. This theory, of course, involves seed transmission of the virus in undetectable form or quantity. The second possible explanation is that the virus is arising spontaneously within the plant. The third possibility, and perhaps the least likely, is the existence of a mode of virus transmission at present quite unsuspected.

Virus workers have long dallied with the idea that a virus might arise *de novo* within the host. Such a suggestion is attractive in some ways and it would explain many things which are at the moment obscure. If viruses are considered as organisms or at least possessing some of the attributes of life, the suggestion of their heterogenesis is repugnant. If, on the other hand, Stanley's view that a virus may be an autocatalytic protein is accepted, then there seems no particular reason why the theory of spontaneous development of the virus within the host should not also be accepted. It is, however, at present still an open question and much work remains to be done before this question can be answered.

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