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Recent Work on Virus Diseases in Plants.

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VIRUS DISEASES occur with remarkable frequency in plants. It would be little exaggeration to say that they may be found in one form or another in every allotment area in England. Their geographical distribution is almost universal, their host-range is wide, and includes many of the valuable commercial crops as well as non-commercial plants found in gardens, fields or hedgerows. They are of great economic importance, being in this country the cause of very serious losses in the potato industry every year, and they have threatened to extinguish the growing of sugar-cane in many localities abroad. At the present time they constitute one of the most important group of disease with which the plant pathologist has to deal.

The effects the diseases produce on the plants vary greatly. Usually they cause a stunting of growth, malformation or distortion of the foliage, with nearly always some chlorosis, and in the case of commercial crops, a reduction of yield. The injury may vary from a mere discoloration of the foliage to total extinction of the plant. The classical sign is a mottling on the leaves, a blotching of lighter green, or yellow, or even white upon the darker background of the green leaf; and when this is a prominent symptom the disease is known as a mosaic. Mosaicing is not an invariable sign, and there is a number of well-known virus diseases in which it may not occur, or occurs only as a subordinate and late secondary symptom—as in *leaf-roll* of potato, in this country one of the serious diseases.

Under natural conditions they are transmitted from plant to plant by insects (usually sucking insects such as aphids, but sometimes biting or crushing insects also [1, 2]). Soil transmission has not been certainly established in any case; nor has transmission by contact; and the problem of control is largely a problem for the plant entomologist. In the laboratory there are three methods of transmission known. The first by grafting, i.e., by establishing organic union between the diseased and healthy tissue. This is the only universal and certain method, and there are some diseases in which no other artificial method has, as yet, succeeded, e.g., *peach yellows*, a disease of peach trees which is commonly fatal. The second is by means of insects, which are allowed to feed on the diseased and then transferred to the healthy plant. There is a group of diseases in which this is the only method known, other than grafting. Leaf-roll of potato is an example. The third is by the inoculation of expressed juice, or crushed tissue, of the infected plant. This is fairly common, especially in the mosaic group, and most of the characters attributed to plant viruses have been worked out in diseases where this is possible—naturally so, since it is only in such cases that the infective material is easily handled and treated. In some cases this form of transmission succeeds with astonishing ease. In tobacco mosaic, for instance, it is enough to rub the leaf of an infected plant between finger and thumb and then rub a leaf of a healthy plant with the same fingers. But in other

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cases, e.g., mosaic of cane-sugar [3] and *curly top* of sugar beet [4], infection is obtained only with the greatest difficulty and in a small percentage of attempts. This difficulty is probably technical, at least in part, and the number of diseases which cannot be transmitted except by grafting or insects is gradually diminishing, as success results with juice inoculation. The history of transmission is, however, curious. When first described it was stated that tomato mosaic could not be transmitted to tobacco [5]; nowadays, this is easy, and rarely fails. Then it was said that a mosaic could be transmitted only within the same family, e.g., a disease in one of the Solanaceæ, such as tomato, was transmissible, perhaps, to other Solanaceæ but not to other plants. This is still true of tobacco mosaic; but in many other cases (tomato, cucumber, Leguminosæ, etc.), cross-inoculation between families has been frequently successful [6]. Whether this is due to improved technique, or to a difference in the viruses now current, one cannot say; but recent literature provides several instances where plants believed to be insusceptible have been successfully infected [7].

It is certain that simple introduction of infective material within a susceptible plant may be insufficient to produce the disease.[1] In tobacco mosaic and other similar diseases, where transmission is effected with the utmost ease by transference of the tiniest drop of juice, one might expect that every insect which fed upon the plant would function as a vector by mere mechanical carriage of the juice upon its external mouth parts, but such is not the case. The large green aphis of the lettuce (*Macrosiphon lactucæ*) feeds, and multiplies readily, on tobacco, but it apparently does not transmit mosaic, although the related insect, *Myzus persicæ*, does so regularly [8 and *cf.* 2, 9]. In other cases, where juice or tissue inoculation fails, or is only exceptionally successful, one may suppose either that the insect introduces the virus into a site at which infection is more readily effective (aphids, for example, usually seek out the phloem-cells with their stylets), or that some favouring substance is contained in the salivary juice, possibly of the nature of an aggressin, or, that the virus undergoes some developmental change within the insect, which is advantageous, or necessary for infection. In *curly-top* of sugar-beet the disease is carried by a jassid, *Eutettix tenella*, and apparently by this insect only; at least nine other insects, found on beets and related plants, have been tried unsuccessfully [9]. It is known that even *Eutettix* will not transmit immediately after feeding on the diseased plant. If the insect is kept at relatively high temperatures (100° F. or over), it can transmit in four to six hours after feeding, but not sooner: and if it is kept at lower temperatures it will not transmit till at least ten hours after feeding, and a higher percentage of success is obtained between twenty-four and seventy-two hours after feeding than at other times [10]. This would indicate that the virus requires to go through some developmental or other change within the insect before it is fully infective, for which there are analogies in animal pathology. The evidence however is not very complete. If mechanical transference is excluded, the virus must pass from the alimentary canal of the insect to the salivary glands before it can be injected, and an interval of four to six hours does not seem very long for this process. Further, sometimes in this disease direct inoculation of juice has proved successful [4], so that the stay in the insect is not essential, although advantageous. More satisfactory is the only other similar case recorded, viz., the transmission of *aster yellows* by *Cicadula sexnotata*, also a jassid [11]. In this disease direct transmission has not yet been achieved, and eight other insects have been tried as vectors without success. The cicadula transmits regularly, but the adult insect does not become infective till six to ten days after feeding; the nymphs also require a long period (not exactly determined but certainly amounting to several days).

[1] This will not seem surprising to the animal pathologist, but it must be remembered that, so far as is known, plants have no defence mechanism, either cellular or humoral, such as is familiar in animals.

That the virus can survive for long periods in the insect is well established; in the case of *Eutettix* for 111 days [4], or during its whole adult life, and the same thing is recorded of the *Cicadula*. Thus *spinach-blight*, a mosaic, is transmitted to the progeny up to the fourth generation [12], possibly farther, which certainly suggests multiplication; but it is not so with *Eutettix* [9], or *Cicadula*.

No visible organism has as yet been isolated with a substantial claim to be the cause of any plant virus disease. Five years ago some sensation was caused by the description of protozoa, alleged to be flagellates and trypanosomes, found in various mosaics and in leaf-roll [13]. This was speedily disproved; but, since then, other and more impressive accounts [14] have been published, from good institutes and by good workers, of flagellates or mycetozoa occurring in virus tissue, and not elsewhere. None of these is established as yet. Olitsky [15] is the only one in recent years to claim success in obtaining multiplication outside the living plant without using any special or unusual technique; he inoculated sterile tomato juice with mosaic tomato juice, and subcultured into sterile tomato juice. He states that he obtained infection with the twelfth subculture, which represented a dilution of the original inoculum of $4/10^{16}$. This has been repeated by other workers [16], including myself, without success. Negative results do not disprove Olitsky's claim, but they indicate that some unnoticed factor must have been present in his experiments, which we have omitted, and which neither he, nor we, have recognized. Microscopic examination of affected tissue by many workers [17] has not yet yielded results. Most observers have found intracellular bodies of various kinds. Thus appearances like Negri or Guarnieri bodies are described, also strongylo-plasma-like bodies or "Elementär-Körperchen," and various types of paranuclear structures. So far they have led to nothing definite. Plant-tissue is a very difficult material for such work, especially when abnormal. It is recognized that they may possibly be reaction-products of the virus itself, and they are not found in every virus disease [18].

The question naturally arises whether these diseases are due to different viruses, or to differing expressions of the activity of a single virus. I do not know what criteria one can lay down to determine with certainty that one virus is, or is not, identical with another. But there is, at least, one case in which two naturally occurring viruses are practically indistinguishable. Tobacco, and tomato mosaic, as they occur naturally, present essentially the same clinical picture and still do so when cross-inoculated. The properties of tobacco virus in tobacco juice and of tomato virus in tomato juice are essentially the same, so far as they have been tested. I do not recall any detailed examination of either virus in the juice of the other host, and no doubt this should be made. But it would probably be hypercritical to deny their identity on that ground alone, and there is general agreement that they are the same. There is a long list of cases in which the symptoms agree, in general, with those of tobacco and tomato mosaic, and in which the properties of the virus, so far as examined, are alike. It is probable that in the mosaic group many diseases are caused by viruses which are indistinguishable from one another.

The importance of comparing the properties of the two viruses, when both are in the same medium or juice, is well shown by a recent experiment of Walker, in Wisconsin [19]. Mosaic in cucumber is very similar to mosaic in tomato, tobacco and ground cherry, and there is a long list of instances of cross-inoculation between mosaic in cucumber and mosaic in other plants, where the diseases so produced are similar to those occurring in the natural host. But it was long supposed that the virus of cucumber mosaic must be different from that of tobacco, because in the juices of these plants they behaved very differently. In tobacco juice, tobacco virus remains infective apparently indefinitely; five years is, I believe,

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the longest period recorded [20] ; it withstands drying—whether air-drying or over sulphuric acid—and precipitation by alcohol (50 per cent. in the case of tobacco [21], but in tomato and ground-cherry 95 per cent. alcohol for one hour did not destroy it; it comes down with the precipitate, and is active when this is redissolved [19]); it is not destroyed by heat up to 80° C. (even two days at this temperature failed in one case to reduce its infectivity [22]), but is usually inactivated in 10 minutes between 85° and 90° C.; it is very resistant to sunlight [22], and to most antiseptics [21]. It may be added that it resists X-rays for half an hour, but is destroyed in one hour by rays from a mercury-vapour lamp [22]. It has also been said to pass through collodion sacs up to a definite grade of fineness of pore [22], but this requires confirmation. Tomato and ground-cherry viruses resemble tobacco virus in all these respects, so far as they have been tested. But cucumber mosaic juice loses its activity in a week on standing, and will not withstand drying, alcohol-precipitation, heating over 70° C., or any antiseptics except toluene or 5 per cent. chloroform [23]. It was therefore believed to be a different virus. Walker showed that when cucumber mosaic was transferred to ground-cherry, and its properties examined in ground-cherry juice, it lost its labile characters and behaved like tomato or ground-cherry mosaic; and, conversely, ground-cherry mosaic transferred to cucumber had acquired the labile characters of cucumber mosaic. The apparent differences, therefore, were simply due to differences in the containing medium or juice, and not to a difference in the viruses themselves. It was concluded, then, that they were identical; and the conclusion is probably correct, though it is a pity that Walker's experiments are not quite so satisfactory as one could wish, owing to some peculiarity of the strain of cucumber mosaic, or cucumber plants with which he worked. If we assume it to be correct, a whole group of mosaics, hitherto believed to be distinct, falls into line with tobacco mosaic. And it may be that all the mosaic diseases will eventually be shown to be due to the same virus. It would not be surprising, since, speaking generally, the clinical picture in the mosaics is of the same type, whatever the kind of plant in which they occur. But, while that is roughly true, there are well-marked differences amongst the mosaics, even in the same plant, which remain distinct on transference from plant to plant, and if the causal agent is the same, some explanation is required for the constancy of these differences.

On the other hand, there are a number of diseases known where the clinical picture is not that of a mosaic. In the curly-top of sugar beets, the prominent feature of the disease is the rolling and curling of the leaves, which is always accompanied by thickening and distortion of the veins on the under surface. The colour changes in the leaf are inconspicuous, and a leaf may show very extensive signs of disease without loss of green. Further, there is an internal definite lesion not found in mosaic, a necrosis of the phloem, often macroscopically visible. The picture is quite unlike mosaic, but is in many respects like potato leaf-roll. Now, on both sugar-beet and on potato, diseases of mosaic type also occur; and each type remains true on transmission from plant to plant, the mosaic not giving rise to curly-top or leaf-roll, nor these to mosaic. Here we have overcome the necessity of cross-inoculation. The problem is in some ways analogous to that of the filtrable tumours, which come true to type on inoculation in the same host. There is no good evidence of a virus disease arising in plants *de novo*. In the early literature there are frequent statements that by cutting back healthy plants, or by manurial treatment, mosaic may be produced [24]. But these statements all date from the time when the importance and frequency of insect transmission were not fully appreciated, and protection from external contamination was not rigorously enforced.

The fact that on the same host one may find two or more diseases clinically quite distinct, and coming true constantly on transmission, is presumptive evidence that the diseases are due to different causes, and the *onus disprobandi* rests with him who would disprove it. There is as yet no evidence that there are in plant virus diseases two factors such as are described in the filtrable tumours.

Up till a short time ago it was believed, on what seemed quite reliable evidence, that it was possible for a species of plant to be immune to a virus disease, in the sense of never showing any signs of the disease, and yet be infective, capable of producing the disease when its juice was inoculated into susceptible species. For example, Allard [25] consistently failed to obtain any signs on inoculating *Nicotiana glutinosa* with tobacco mosaic; nor, when a susceptible variety was grafted on to it and this susceptible scion inoculated, did the *glutinosa* develop signs, although the scion took the disease. Again, Quanjer [26] grafted on a healthy tomato plant the top of a leaf-roll potato; the tomato showed no signs of the disease, but, if the potato graft were removed and a part of the apparently healthy tomato in turn grafted on healthy potato, the latter developed leaf-roll. Both the tomato and the tobacco were apparently immune, and yet capable of transmitting. But now, I think, plant pathologists would be more guarded in their statements. In several such cases as those I have quoted it has been subsequently shown that the carrier species was not really immune. *Nicotiana glutinosa*, for example, has been shown by two independent workers [27] to be really a species susceptible to tobacco mosaic, though Allard failed to produce infection; and it is doubtful whether there is any sure case of a genuinely immune variety carrying infection. There is, however, no doubt that a plant may be infectious at a time when it is not showing signs, and that carriers in this sense exist.

Much evidence has accumulated as to the effect of external circumstances on the development of symptoms [28]. A potato with well-marked mosaic loses the signs of the disease on transference for a week to a temperature of 24° C. or higher; and an inoculated plant kept at this temperature develops no symptoms. No such effect occurs at temperatures below 20°, but above this limit the higher the temperature the more rapidly does masking of symptoms occur. The exposure need not be continuous: intermittent exposure produces the same effect, but it takes longer. The plant is not cured; on removal to lower temperatures the signs reappear, and new leaves developing are mottled. For tobacco, the temperatures necessary are higher, viz., 36° to 37° C. Further, there is a range of temperature that is most favourable to the development of signs: in tobacco 28° to 30°, in potato 14° to 18°. This favourable temperature is no doubt conditioned to some extent by the temperature which gives the best growth of the plant. It is a familiar observation that in many virus diseases signs appear soonest and are most marked on young, actively growing parts of the plant, e.g., the new leaves, while leaves which were already fully grown before infection occurred may show no signs, although their sap is infective. And there are many observations scattered through the literature, that when a plant is growing slowly (as in winter), virus symptoms may not appear, or only after unusually prolonged incubation. But it is not yet quite clear whether this partial suppression is due to the slowness of the growth or to lower temperature, or to both combined.

Observations of this kind have led to the belief that virus can develop only when growth is taking place, i.e., that multiplication occurs only when cell-division is in progress. In potatoes, for example, if infection occurs late in the season, when the plant has reached its full growth, signs will not develop that year. In cross-inoculation, too, between families, or in the cases where juice-transmission is difficult, success is more frequent if the plants are young and actively growing.

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But the evidence is not sufficient to establish this very important fact. It does not really amount to more than that signs appear most markedly when active growth is in progress, and may not appear when growth is slow or at a standstill; and, as we have just seen, development of signs is not a necessary consequence of development of virus. Even in the late-infected potato, the virus multiplies, since the tubers are infected, and produce infected plants the next year. But of course, so long as a plant remains alive, it is hardly possible to say that no cell-division is occurring.

A remarkable phenomenon, which may have a bearing on the carrier-question, has recently been described by Johnson [29]. He has found that the foliage of normal, apparently healthy potatoes, when crushed and inoculated into perfectly normal tobacco plants, produces in the latter a virus disease. At first the symptoms are very slight in the tobacco, and are unlike ordinary mosaics in that they appear first, not on the youngest leaves, but on the older leaves of the plant. On later transfers from tobacco to tobacco, however, the signs become much more marked; they are accompanied by stunting and malformation, and the mottled areas become, in some instances at least, necrotic.[1] There would seem to be at least two, possibly three, types of disease so produced; but the main point that concerns us at the moment is that inoculation of healthy foliage produces virus disease. That it is a virus is shown by its filtrability and its resistance to high dilution; it is transmitted by aphids; is usually short-lived (less than three weeks) outside the plant, and is destroyed in ten minutes at 70° C. Other solanaceous plants (eight have been tried) respond to potato in the same way as tobacco, but of eighteen other species of plants tried, none produced a virus disease in tobacco. Amongst potatoes the property seems to be general. Ten different standard varieties of potato, brought from the most widely separated regions of North America, gave successful results; but the most consistent results were obtained with the variety "Triumph," where from 170 separate plants 965 inoculations were made to tobacco, with 80 per cent. of success. The fact seems undeniable: the only question is that of interpretation.

It is admittedly rather troublesome in the case of potato to determine that any given plant is free from virus disease. At least seven, possibly more, different diseases have been distinguished by the symptom-complex. Of these some differ only comparatively slightly from one another, but there are at least three (leaf-roll, mild mosaic, and crinkle) in which the clinical picture is very distinct. Further, as we have seen, the potato may show no signs in the year in which it is infected, so that it is necessary to grow the tubers of each plant the following year in order to be sure that it is free from disease. All precautions, however, were taken in Johnson's experiments, and the plants were normal in the ordinary usage of the term. Yet, on inoculation they produce a virus disease.

Naturally, the obvious explanation is that the potato carries a virus in a masked state, and this is supported by the fact that with material from seedlings grown

[1] This would seem to be a clear case of increase of virulence on passage, and is the only instance recorded for a plant virus. Attenuation of virus by passage through resistant plants has been described for the curly-top of beets [30]; and in the case of tobacco by growing infected plants at high temperatures (ten days at 35° to 37°). In the latter case the virus remains attenuated even after repeated passage in tobacco under normal conditions [31].

from true-seed (only a small number were tested) no success was forthcoming on inoculating tobacco. But it is a little difficult to accept this explanation without hesitation. It would mean that the majority of potatoes, of whatever variety and whatever their source, are carriers of one or more diseases, which produce no signs in them under any conditions to which they have yet been subjected; in fact, that the potato plant is normally a carrier. Further, when the diseases are transferred back again from tobacco to potato, one at least of them produces a disease so malignant as sometimes to kill the plant in fifteen to thirty days. If, then, the original potato was carrying the virus without signs, the virus has been so altered by its sojourn in tobacco as now to be virulent for the potato, where formerly it was avirulent. This, of course, is not impossible, and, as we have seen, its virulence was raised by passage in the tobacco. Also, the re-transferred disease, at first so virulent for potato and tobacco, loses something of its malignancy for both on repeated subculture in the potato, though it does not become avirulent, or has not yet become so.

A similar phenomenon has since then been recorded: this time between potato and potato [32]. One variety of normal potato (Green Mountain), when transferred to other varieties by grafting or juice inoculation, produced in some of them, though not in all, a necrotic lesion without mottling, somewhat resembling a recognized virus disease of potato called *streak*. The Green Mountain variety of potato is the only one yet known to be capable of producing this result, which cannot be identical with streak, since Green Mountain is susceptible to streak.

Perhaps the easiest interpretation of these facts is to suppose that potato is a plant which normally carries suppressed disease, difficult as that view may be. But the facts have given a new stimulus to the school [33] which believes that virus diseases are not due to any organism or parasite at all, but that a virus is to be conceived as a product of a plant's own metabolic activity under the influence of some disturbing factor, viz., the virus material itself, for which the name of *viroplasm* has been coined.

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