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On the Interactions of two Strains of a Plant Virus; Experiments on Induced Immunity in Plants

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[PLATES 14–16]

INTRODUCTION

The nature of the causative agent of virus diseases is obscure. A great deal of information on the reactions of the agent and on the symptomatology of these diseases has, however, been accumulated, with a view to bringing to the solution of the main problem the largest available amount of detail. While there is no irrefutable argument in support of either the organismal or the non-organismal hypothesis, it has become clear, as the investigations have progressed, that the viruses of both plant and of animal diseases possess many of the qualities usually associated with organisms. The existence of strains in the animal viruses is now generally accepted, though less information has been available regarding this aspect of the plant viruses. The purpose of the present paper is to direct attention to the presence of strains in what was previously thought to be a single virus, and to the probability of similar conditions obtaining in other viruses. The interactions of these strains, one with another, and with other viruses have been examined and are also described and discussed.

It is shown that four types of interaction between different plant viruses can be recognized and these types are examined in some detail.

MATERIALS AND METHODS

The virus which was used in the study of strains was that of "Aucuba" or Yellow Mosaic of tomato. This virus has been intensively studied in this laboratory for some years and there is good evidence for supposing that it is identical with the *Tobacco Virus No.* 6 of Johnson, the causative agent of Yellow Mosaic of tobacco. Yellow Mosaic of tomatoes has been known in this country for some years, and was first described by Bewley (1924), the causative agent being studied in some detail by Henderson Smith (1928). The viruses were studied in their host plants

tomato (*Lycopersicum esculentum* var. " Kondine Red "), tobacco (*Nicotiana tabacum* var. " White Burley "), *Nicotiana glutinosa*, and *Solanum nodiflorum*.

Henderson Smith described the virus as being " possibly identical with Johnson's *T.V. No.* 6 which differs from that of ordinary tomato mosaic in the brilliance and intensity of its leaf symptoms, but in other respects is indistinguishable from it by the characters investigated." These characters were its longevity, its heat resistance (it is not destroyed by exposure to 80° C for 10 minutes), its resistance to alcohol up to 90% and to other chemicals, and its infectivity in diluted juice. The symptoms of the disease are described as follows: " In extreme cases almost the whole of the surface is pale yellow to white with here and there small islets of intense dark green which stand up as small blisters. In less extreme cases, the green areas are larger, but as a rule the area of white or pale yellow is greater than the green area . . . Scattered over the leaf are patches of white and patches of yellow, usually sharply delineated, but sometimes shading into neighbouring areas, irregular in shape and size, often angular and occurring in all parts of the leaf . . . The plant is not killed . . . There is no necrosis in tomato, . . . There is little tendency to extreme malformation though quite definite " fern-leaf " has been noted on plants growing rather slowly, *e.g.*, in the autumn . . ."

To this description some further details may now be added. The virus attacks a wide range of hosts, particularly among the *Solanaceæ* and in many it produces symptoms very similar to those described for tomato. Further, in some hosts, notably *S. nodiflorum*, it induces the formation of " X " bodies in the cells. The large spherical inclusion bodies induced by this virus in *S. nodiflorum* are characteristic of the disease and may be used for diagnostic purposes, being readily distinguished from the smaller inclusion bodies typical of other viruses in this host.

From the observations made in this laboratory on Yellow Mosaic of tomato since the publication of the paper cited above, it was concluded that the disease was characterized by two main groups of symptoms. In summer, when plants are growing well and light intensity is high, the symptoms were as described above. In winter, on the other hand, and when the growth of the tomato plants was slow, the chlorosis was much less marked and was often indistinguishable from the indeterminate mottle which is characteristic of the tomato Mosaic caused by *T.V. No.* 1 of Johnson (1927). It was therefore assumed that there were " summer " symptoms, formed in conditions of high light intensity, and " winter "

symptoms which occur when the light intensity is low and the growth of the plants reduced.

It was not until the spring of 1933 that evidence of the existence of strains in the virus presented itself. It was then noticed, after a spell of particularly sunny weather, that individual plants of a group of tomatoes inoculated with a culture of Yellow Mosaic virus, which had been kept in our glasshouses since 1925, showed unusual symptoms. Some were only faintly mottled as is typical of very mild " winter " symptoms, while others showed the most marked chlorosis with intensely bright yellow areas on the leaves; the majority occupied an intermediate position as regards symptom intensity. In this instance, at least, light intensity was evidently not the controlling factor in symptom expression, since three distinct groups of symptoms were obtained under the same environmental conditions. The existence of different strains of the virus, possibly an attenuated and a more virulent form, as have been shown to exist in *T.V. No.* 1, was, therefore, suspected. With a view to examining this possibility inocula were prepared from single plants showing the typical symptoms of each group and were inoculated into healthy tomato seedlings. After an appropriate interval symptoms of the same types were developed in the inoculated plants. Cultures were made from the two extreme groups, the mild type of symptoms being for convenience called " green " Mosaic and the more severe types being called " yellow " Mosaic. These were the materials with which the experiments now to be described were carried out.

The " Green " Material—To ensure that no contamination of the stock material had taken place, the juice of diseased tomato plants showing the " green " type of symptoms was submitted to the standard treatment *in vitro*, to determine the physical properties of the virus responsible for the development of symptoms. The properties were those characteristic of the virus of classical " Aucuba " material, *i.e.*, *T.V. No.* 6. This did not preclude the possibility of the virus being *T.V. No.* 1, however, which has identical properties *in vitro* with *T.V. No.* 6. A simple test with tobacco plants is sufficient to distinguish between these two viruses, since *T.V. No.* 6 normally causes the formation of necrotic spots on the rubbed leaves of tobacco, while *T.V. No.* 1 causes only faint chlorotic spots or no visible symptoms on the rubbed leaves. Two groups of young tobacco plants were, therefore, inoculated one with *T.V. No.* 1 juice, the other with juice from a plant with " green " Mosaic. In the former group there were no local necrotic lesions on the rubbed leaves, the first symptoms being the systemic chlorosis typical of tobacco Mosaic, while in the

latter group local necrotic lesions appeared on the rubbed leaves on the third day after inoculation. It is clear, therefore, that the " green " Mosaic virus is not identical with *T.V. No.* 1 and for convenience it is designated subsequently as A.G. virus in this paper.

After this point had been established, serial inoculations of the A.G. material were made at intervals of three or four weeks into groups of tomato plants, care being taken to avoid contamination and to examine the plants for the appearance of the usual symptoms of tomato Yellow Mosaic. After a large number of such transfers it was found that the A.G. virus was quite consistent in its action in the host plant and a series of experiments with other hosts was set up. The symptoms found in the different hosts were as follows:—

(*a*) *In Tomato*—A faint generalized mottle with little leaf distortion and little stunting of the plant. Symptoms appear some 10 to 15 days after inoculation.

(*b*) *In Nicotiana glauca*—No necrotic local lesions (inoculations were made by the pin-prick method since there is an almost complete absence of hairs on the leaf of this plant). A faint mottle visible on the upper leaves of some plants a few weeks after inoculation, but, more generally, no apparent symptoms.

(*c*) *In Solanum nodiflorum*—No necrotic local lesions—and systemic symptoms a faint mottle with little leaf distortion or stunting of the plant. Symptoms appear some 12 to 15 days after inoculation. The inclusion bodies in the infected hair-cells are quite typical of " Aucuba " or Yellow Mosaic of tomato.

(*d*) *In Nicotiana tabacum* (var. " White Burley ")—Marked necrotic local lesions on the rubbed leaves followed by a systemic chlorosis with or without necrosis (occasionally causing death of young plants).

(*e*) *In Nicotiana glutinosa*—Marked necrotic local lesions on the rubbed leaves. No systemic infection.

(*f*) *In Zinnia sp*—No apparent local lesions on the rubbed leaves. Faint mottle on older leaves some 14 days after inoculation.

The " Yellow " Material—The " yellow " material was treated in a similar manner. Serial transfers from group to group of tomato plants led to the conclusion that this virus gave consistent results in this host. It was, therefore, designated A.Y. virus for convenience. In all its reactions *in vitro* this virus is identical with the A.G. virus above described and

in the next section evidence is adduced to show that they are, in fact, strains of the same virus, viz., *T.V. No.* 6. It is further suggested, as a result of observations detailed later, that the disease as it occurs in the field is usually caused by a mixture of these two strains.

The symptoms induced by the A.Y. virus in the same host plants as were used for the A.G. virus are as follows:—

(*a*) *In Tomato*—A bright yellow mottle with some leaf distortion and stunting of the plant. Symptoms appear from 5 to 20 days after inoculation. In bright sunny weather the incubation period is shorter.

(*b*) *In Nicotiana glauca*—No necrotic local lesions—inoculations were made by the pin-prick method since there is an almost complete absence of hairs on the leaf of this plant. Bright yellow symptoms appear on the young leaves after some 10 days. The mottle often takes the form of concentric rings. There is little distortion or stunting of the plant or leaves and no necrosis.

(*c*) *In Solanum nodiflorum*—No necrotic local lesions. Occasionally chlorotic local lesions on leaves rubbed when young. The systemic symptoms, as in tomato, are a bright yellow mottle. There is no necrosis. The symptoms appear 7 to 20 days after inoculation. The inclusion bodies in the infected hair-cells are quite typical in this mosaic.

(*d*) *In Nicotiana tabacum* (var. " White Burley ")—Marked necrotic local lesions developed on the rubbed leaves, followed by a systemic chlorosis with or without necrosis (occasionally, in the former instance, causing the death of young plants). The symptoms induced by the A.Y. virus strain in this plant are very similar to those caused by the A.G. virus strain.

(*e*) *In Nicotiana glutinosa*—Marked necrotic local lesions on the rubbed leaves. No systemic infection.

(*f*) *In Zinnia sp.*—Occasional chlorotic local lesions on the rubbed leaves. Bright systemic mottle irregularly on younger leaves of plant after some 10 days incubation.

A series of photographs of the plants, Plates 14–16, illustrate clearly the marked differences in the symptom-picture induced by the two strains of the virus.

The Identity of the Virus Strains

A detailed examination of the two strains was made to discover whether they are actually strains of the same virus or two closely related viruses.

Many of the symptoms induced by the A.G. virus, for example, might equally well have indicated the presence of a mixture of two viruses, viz., *T.V. No.* 1 and *Tomato Streak Virus No.* 1. Those two viruses have reactions *in vitro* identical with those of *T.V. No.* 6 and are separable from it only through differential hosts. *T.V. No.* 1 induces no necrotic local lesions in tobacco but only a systemic chlorosis, while *T.S.V. No.* 1 induces local necrotic lesions and no systemic chlorosis. Should systemic symptoms occur with the latter virus, they take the form of severe necrosis, which often kills the plant. If, therefore, a mixture of the two were inoculated into tobacco, it would induce local necrotic lesions, followed by a mild systemic chlorosis. This is similar to the symptom-picture of the A.G. mosaic. A second inoculation, from the upper leaves of tobacco plants infected with a mixture of *T.V. No.* 1 and *T.S.V. No.* 1, made into tobacco, would serve to demonstrate the absence of *T.S.V. No.* 1 in those upper leaves, since no local necrotic lesions would result. Inoculations into tobacco, from the upper leaves of tobacco plants infected with A.G. virus, indicated, by the development of local lesions in the rubbed leaves, that this was not a mixture of the *T.V. No.* 1 and the *T.S.V. No.* 1 viruses.

Further evidence on this point was furnished by a study of the leaf hairs of *S. nodiflorum*. In the cells of this plant are developed the characteristic inclusion bodies of the Yellow Mosaic disease. These have been described in some detail by both Henderson Smith (1930) and Sheffield (1931), working in this laboratory. Inoculations of A.G. virus material were made into one group of *S. nodiflorum* plants while inoculations of a mixture of *T.V. No.* 1 and *T.S.V. No.* 1 were made into a second group. After a fortnight, the inclusion bodies in the cells of both sets of plants were examined. The cells of the leaf-hairs of the plants inoculated with the A.G. virus contained the usual inclusion bodies ("X" bodies) typical of the disease, while those of the other plants had inclusions of a different type, characteristic of *T.V. No.* 1.

Infections from the upper leaves of the plants infected with the mixed viruses were made into young tobacco plants. It was found that both viruses had multiplied in the tissues of the *S. nodiflorum* plants as the inoculated leaves of the tobaccos developed necrotic local lesions typical of the *T.S.V. No.* 1, while the later appearance of typical systemic chlorosis indicated the presence of *T.V. No.* 1.

All the available evidence, therefore, points to the view that these two viruses A.G. and A.Y. are, in fact, strains of the virus known as *T.V. No.* 6.

The Effect of Inoculating the Two Strains into the same Host Plant

A series of experiments were set up in which the two strains were mixed together and inoculated into young tomato plants. Both are easily inoculated by rubbing the host plant with infected juice and both multiply readily in the tissues; there is no evidence that one is more virulent than the other.

The question of concentration of the viruses does, however, arise in this connection and an examination was made of the amount of virus present in the tissues of similar tomato plants inoculated with each of the strains separately and with a mixture of both strains. A very fair measure of the amount of the virus present may readily be made by counting the necrotic lesions which follow the inoculation of known amounts of extracted juice on the leaves of *N. glutinosa*, in which plant, as has been shown (Chester, 1933) both strains induce the same symptoms, viz., necrotic local lesions on the rubbed leaves (Caldwell, 1933). When similar amounts of diseased tissue, macerated in water, were inoculated on to the leaves of *N. glutinosa*, no difference was found in the concentration of the virus in tomato plants inoculated with the A.G. strain, the A.Y. strain, or with a mixture of both.

In the first group of experiments on inoculation with the mixture of the two strains A.G. and A.Y., the two materials were mixed in equal proportion and inoculated into young tomato plants. After an appropriate interval symptoms of normal Yellow Mosaic appeared on the young leaves. The symptoms appeared to be intermediate in intensity between those of the A.G. form and of the A.Y. form. Neither strain was, apparently, able completely to inhibit the development of the other under the environmental conditions obtaining during the experiment.

When mixtures in which one of the components was in considerable excess were used as inocula the symptoms developed in the tomato plants tended, in the main, to approximate more nearly to those associated with the major component.

The symptoms caused by the A.G. strain clearly could not be detected in a plant previously inoculated with the yellow strain, but the converse does not hold. The intensity of the symptoms caused by the yellow strain is so much greater than that of the symptoms of the green that they can be easily recognized in the presence of the latter. A series of experiments was therefore, set up to discover the effect of inoculating a plant already infected with A.G. virus with the A.Y. strain.

A group of tomato plants were inoculated with the A.G. strain and

after a fortnight showed symptoms typical of this virus. Thereafter, further inoculations of A.Y. material were made into the same plants, but no symptoms of Yellow Mosaic were developed. This has been repeated many times and so far, 18 months after the isolation of the original cultures of the strains, no instance has occurred in which the A.G. strain has failed to immunize plants against the A.Y. strain. This immunity is apparently complete in that it is not possible to recover from plants which have been inoculated with the A.G. and then with the A.Y. strain any trace of the A.Y. strain. Many attempts have been made involving a large number of plants and the immunity is not only expressed in the suppression of symptoms, but also in the prevention of development of the second strain within the tissues.

This immunity is rapidly set up in the tissues as attempted infection of tomatoes with the A.Y. strain three or four days after inoculation with the A.G. strain has been consistently unsuccessful. If the second inoculation be made into the younger leaves before the first virus has left the inoculated leaf, which is usually some 48 hours or so after inoculation, then symptoms of the " mixed " type are usually found.

Immunity against the A.Y. Strain induced by the A.G. Strain in *Zinnia sp.*

The complete immunity against the A.Y. strain which is induced in tomato by a previous infection with the A.G. strain of virus is found also in *Zinnia sp.* Groups of plants were inoculated with A.G. strain and were allowed to develop the very mild symptoms characteristic of the disease. Thereafter one-half of the plants were inoculated with A.Y. strain as were also a group of healthy controls. The controls all developed marked symptoms of Yellow Mosaic after some 10 days, while the other plants did not. At the end of a fortnight the top leaves of plants from each group were inoculated into groups of tomato in which the tissues from the Zinnias infected with A.G. strain produced the usual green symptoms, as also did those which had been inoculated with both strains, while the leaves of the plants with the yellow strain produced the usual marked symptoms of Yellow Mosaic. In *Zinnia*, therefore, there is apparently complete immunity against the A.Y. strain induced by the A.G. strain of virus.

The Appearance of Summer and Winter Symptoms of Yellow Mosaic

Mention has been made of the fact that the A.Y. strain appears to have a variable incubation period, depending on the light intensity at

the time of inoculation. During the winter months plants in our glasshouses were kept under 1000-watt lamps at a distance of 3 to 4 feet for some 6 hours each night to make up for the deficiency of light during the day. Inoculations were made with both strains and tomatoes so infected showed the symptoms typical of the appropriate strain. The " green " symptoms were no more intense under the lights than they were in plants grown under normal conditions.

The fact that the two strains retain their identity over long periods was shown by a set of experiments on filtration. The juice of plants infected with the A.Y. and with the A.G. strains was passed through filter paper impregnated with fuller's earth and then through L1 and L3 Pasteur-Chamberland filters. The sterile juice so obtained was kept for 6 months in sealed tubes under aseptic conditions, and the juice then inoculated into tomato and *N. glutinosa* plants. The A.G. strain induced the formation of " green " symptoms, the A.Y. strain of " yellow " symptoms—similar to those on the plant from which the virus material had been obtained. Another sample of each juice was kept for a year under similar conditions and gave similar results on inoculation after that interval.

The existence of these two strains in the virus of Yellow Mosaic, and the possibility that there may be more strains not yet identified, offer a probable explanation for the differences in the summer and winter symptoms induced by this virus on tomato. The green strain appears to have the shorter incubation period under winter conditions while the yellow has the shorter under summer conditions. If one assumes that the virus as obtained from the field has some of these and probable other strains present in it, then, in the dull cold conditions of winter, the green will tend to spread more rapidly than the yellow strain, giving the winter symptoms, whereas the reverse would hold in summer. This phenomenon has, in fact, been observed. A stock of Yellow Mosaic had been kept in our glasshouses in tomato plants grown, as a precautionary measure, under muslin cages; at various intervals, material was taken from these plants and used for experimental purposes. In the spring of this year it was noticed that the stock plants had ceased to show the bright yellow symptoms characteristic of the disease and were only faintly mottled—they did show symptoms characteristic of the A.G. strain. When inoculations were made from these plants into healthy plants of tomato, and secondary inoculations with A.Y. strain were made after an interval of 10 days, the yellow symptoms characteristic of the second strain did not appear in any of the plants. It is suggested that the effect of keeping the plants under muslin was to maintain their metabolism at the " winter "

rather than the " summer " level and that, in consequence, over a long series of transfers, the " green " strain of virus had been encouraged at the expense of the " yellow." In the earlier samples enough of the " yellow " strain had been left to allow of its multiplication under normal summer conditions.

In some virus diseases (Salaman, 1933) isolated portions of the infected plants appear to contain different components of the virus complex. The virus under discussion does not appear to belong to this group. The tiny yellowish flecks which occur infrequently on the leaves of plants infected with A.G. strain have been punched out of the leaves with a punch made from narrow-bore brass tubing, to preclude the removal of other portions of the leaf at the same time. Inoculations with these isolated portions have invariably been followed by the development of typical symptoms of the A.G. strain in tomato plants. Similarly, inoculations with portions of the green tissue from leaves infected with A.Y. strain are always followed by the development of the normal symptoms of the A.Y. strain. Specific cells of the host plants infected with a mixture of these two strains apparently become infected with one or other virus-strain, but there is no evidence that substantial portions of the lamina are infected with any one strain to the exclusion of the other.

The Immunity against Yellow Mosaic Virus Induced by other Viruses

When the complete immunity to the A.Y. strain, induced in plants by infection with the A.G. strain of tomato Yellow Mosaic virus, had been clearly established an examination was made of the immunity, if any, induced by other viruses against the same virus. The viruses examined and the results obtained are detailed in the succeeding paragraphs.

(*a*) *The Interaction of Tobacco Mosaic and Tobacco Yellow Mosaic Viruses*—The first reaction examined was that of *T.V. No.* 1 with the strains A.Y. and A.G. This virus is the agent of the commonest of all tobacco mosaics and, as has been seen, differs from that of tobacco Yellow Mosaic only in the intensity of the symptoms produced in tomato, and in some other hosts, being indistinguishable from it as regards reactions *in vitro*. There may, therefore, be some close affinity between these two viruses, especially as there have been suggestions that a yellow form of tobacco Mosaic may arise in plants infected with *T.V. No.* 1 (Jensen, 1933). A group of six tomato plants were inoculated with *T.V. No.* 1 and after a week they showed marked symptoms of ordinary Mosaic of tomatoes. They were then inoculated with the A.Y. material

as were also another group of tomatoes of the same age, which being previously healthy served as controls. A few days later the control plants developed the typical symptoms of Yellow Mosaic, while no trace of this disease was evident in the doubly inoculated plants. After a fortnight, which is three times the maximum incubation period for Yellow Mosaic in summer, the top leaves of the infected plants were removed and macerated with water.

A simple diagnostic difference between these two viruses is the appearance of necrotic local lesions on the leaves of tobacco rubbed with *T.V. No.* 6 (the agent of Yellow Mosaic). The macerated material prepared from the tops of the doubly-inoculated plants was therefore rubbed on to leaves of tobacco plants. No necrotic local lesions appeared on any of the inoculated leaves. Systemic symptoms of ordinary tobacco mosaic did develop, however, after an appropriate interval.

In order to ascertain whether *T.V. No.* 1 might have an inhibitory effect on *T.V. No.* 6 which might prevent the appearance of symptoms on the rubbed leaves of tobacco, in the presence of the first virus, a mixture of juices containing the two viruses was inoculated on to leaves of tobacco. Normal necrotic local lesions developed within 3 days. It is clear, therefore, that the presence of *T.V. No.* 1 does not prevent the appearance of the necrotic lesions induced by *T.V. No.* 6.

In the tops of the plants, which had been doubly inoculated as described above, no multiplication of *T.V. No.* 6 had taken place. The immunity against *T.V. No.* 6 induced in the tomato by *T.V. No.* 1 is apparently complete, and not only are no symptoms produced, but the development of the virus is also inhibited. The A.Y. virus was clearly available to the experimental plants, as there was 100% infection of Yellow Mosaic in the controls, in these and in other experiments.

Similar groups of experiments were set up with the A.G. strain and *T.V. No.* 1. Tomato plants were inoculated with the latter virus and, after symptoms had developed with the former. After a fortnight or three weeks the tops were removed and macerated in water. No attempt was made to distinguish symptoms indicative of A.G. virus against those of *T.V. No.* 1 as the A.G. virus symptoms are less conspicuous than the others. Four groups of tobacco plants were inoculated, the first with the material from the top leaves of the tomato plants above mentioned, the second with a mixture of juices containing *T.V. No.* 1 and A.G. strain virus, the third with *T.V. No.* 1 material alone and the fourth with A.G. strain material alone. No necrotic local lesions were formed in the first and third groups, but the normal lesions characteristic of *T.V. No.* 6 were found in the leaves of the plants of the second and fourth groups.

T.V. No. 1, therefore, induces in tomato plants as complete an immunity against A.G. strain virus as it does against the A.Y. strain of the same virus. No development of either strain, apparently, takes place in tissues infected with *T.V. No.* 1.

(*b*) *The Interaction of the Virus of Valleau's Ring Mosaic and the A.Y. Strain*—The virus of Valleau's Ring Mosaic (Valleau and Johnson, 1930) causes a disease of tobacco, which as its name suggests, is characterized by the appearance on the leaves of chlorotic rings. Under the conditions in our glasshouses the disease symptoms in tomato are not very well marked, consisting of necrotic lesions on the stems, leaves, and petioles. Very occasionally the necrosis may be severe, the condition which is common, apparently, in America.

Groups of tomato plants were inoculated with the virus of Valleau's Ring Mosaic (subsequently called V.R.M.) and after some 10 days when the first symptoms of the disease had appeared they were inoculated with the juice containing the A.Y. strain. No symptoms of the Yellow Mosaic had appeared on the plants after 4 weeks, although the inoculated controls showed the usual symptoms on the fifth or sixth day. Occasional chlorotic areas did appear on some of the plants some 6 weeks after the second inoculation.

The V.R.M. virus differs from that of Yellow Mosaic in that the latter survives heating at 80° C for 10 minutes while the former is destroyed at that temperature. The upper leaves of plants inoculated with both viruses were removed 4 weeks after the second inoculation, were macerated with water and were inoculated on to the leaves of *N. glutinosa* plants. Necrotic local lesions developed on the rubbed leaves, but since both viruses induce these symptoms, it is not possible to separate them in this way. Another portion of the same juice was heated at 80° C for 15 minutes, was cooled, and inoculated on to other leaves of *N. glutinosa* plants. In this instance no necrotic lesions appeared. The A.Y. virus had therefore not reached the upper leaves of the doubly-inoculated plants in less than 4 weeks. As has been noted, some of the doubly-inoculated plants did, as they grew older, show symptoms of Yellow Mosaic and from the upper leaves some A.Y. virus was recovered. This has been tested out on *N. glutinosa* on tobacco and on tomato. The concentration of virus in them, however, was very low and multiplication of the A.Y. virus is much reduced in these plants in which it is not completely inhibited by V.R.M. virus.

(*c*) *The Interaction of "Streak" Virus with the A.Y. Virus*—There occurs in the commercial glasshouses in this and in other countries a

disease of tomatoes known as " Streak." One form of this disease is caused by a single virus known as *Tomato Streak Virus No.* 1 (*Tom. S.V. No.* 1), and it is with this virus that these experiments were carried out. This virus causes two types of disease in the tomato; one is necrotic and is characterized by the presence on the leaves and petioles of necrotic lesions, often scar-like (hence the name " Streak "). The other type is purely chlorotic and is not unlike, in symptoms, the diseases caused by *T.V. No.* 1, viz., ordinary tomato Mosaic. Tissue from plants showing either form of the disease may, on inoculation, induce in healthy tomato plants either form of the disease in different plants of the same batch, though what factor conditions the type of disease symptoms which will develop is not as yet known.

Groups of tomato plants were inoculated with " Streak " virus and after some 10 days, when the symptoms of the disease had appeared, with A.Y. virus. Groups of healthy plants were also inoculated with the A.Y. strain. After a fortnight, many days after the inoculated controls showed symptoms of Yellow Mosaic, the doubly-infected plants were examined and were found to show no symptoms of Yellow Mosaic.

Tom. S.V. No. 1 and the A.Y. strain may be distinguished by the fact that the former induces in tobacco necrotic local lesions followed usually by no systemic infection, or occasionally by systemic necrosis, while the latter, as has been seen, induces necrotic local lesions followed by systemic chlorosis. The appearance, therefore, in inoculated tobacco plants of necrotic local lesions, would not distinguish between the two viruses, whereas the subsequent development or non-development of systemic chlorosis would.

The upper leaves of plants which had been inoculated with both viruses were, therefore, macerated in water and the extracted juice was inoculated on to the leaves of young tobacco plants. After an interval of 4 days, the rubbed leaves showed marked necrotic lesions while after 7 to 10 days, a proportion of the plants showed systemic chlorosis, with or without necrosis, and when juice from their upper leaves was inoculated into young tomato plants Yellow Mosaic symptoms appeared. The effect of the presence of the " Streak " virus was, therefore, to inhibit the appearance of the symptoms of Yellow Mosaic in the doubly-inoculated plants, though the presence of *Tom. S.V. No.* 1 in the tissues does not, apparently, completely inhibit the development of A.Y. virus inoculated secondarily.

(*d*) *The Interaction of Potato " X " Virus with the A.Y. Strain*—The potato Mosaic Virus " X " has been studied at some length by various workers

and numerous strains have been isolated. It is readily juice transmitted and in the tomato induces a disease " Spot Necrosis " which is a systemic chlorosis, with or without tiny necrotic spots. When this virus is inoculated into tomatoes together with a tobacco virus the disease caused is a very severe necrosis—" Experimental Streak " (Dickson, 1923). The " X " virus is less stable than are the tobacco viruses and is destroyed by heating the diseased juice at 70° C for 10 minutes. Groups of tomato plants were inoculated with " X " virus and after 10 days, when the disease symptoms were clearly defined, the plants were inoculated with the A.Y. strain. After 5 days every plant developed the most marked necrosis characteristic of " Experimental Streak," which indicated, not only that the " X " virus had in no way inhibited the development of the A.Y. strain in the infected tissues, but that the two viruses together induced a more severe disease than either alone. Material from the tops of the plants was macerated with water, filtered, heated at 80° C for 15 minutes and inoculated into young tomato plants where, after an appropriate interval, the typical symptoms of Yellow Mosaic developed. In this instance, the presence of the first virus in the tissue had not acted as a preventive, but had, in fact, resulted in the development of more severe symptoms after the second infection than would have been induced by either of the viruses acting alone.

Similar results were obtained when the A.G. strain was used in conjunction with the " X " virus.

(*e*) *The Interaction of " Spotted Wilt " Virus with the A.Y. Strain*—The virus of the tomato disease " Spotted Wilt " is unlike any of the other viruses used in these experiments as it is destroyed by exposure to a temperature of 45° C for 10 minutes, whereas the other viruses are relatively thermo-stable. The other viruses are also fairly resistant to ageing, while the " Spotted Wilt " virus is inactivated after a few hours' exposure at room temperature. The symptom picture in the tomato of this disease is quite unlike that of Yellow Mosaic, being bronzing of the leaves, marked stunting of the plant, and some necrosis.

Groups of tomato plants were inoculated with this virus and showed signs of infection within 5 days. The effect of the first infection was so marked and the disease symptoms so severe that the plants made practically no growth, having been inoculated as fairly young plants. For this reason they were allowed to grow for some 5 weeks before an inoculation with A.Y. virus was made. Symptoms of Yellow Mosaic appear on the doubly-inoculated plants rather slowly, the delay being due, it is believed, to the slow growth of the plants rather than to the inhibition of

the development of the virus by that of " Spotted Wilt." The upper leaves of the plants were macerated with water and used as an inoculum. This juice was left overnight on the laboratory bench and was heated at 70° C for 10 minutes before inoculation into young tomato plants. Either of these treatments is sufficient to destroy the " Spotted Wilt " virus. The inoculated tomato plants developed typical symptoms of Yellow Mosaic, showing that the two viruses in this instance develop independently and that each induces the formation of the appropriate disease symptoms.

(*f*) *The Interaction in Tobacco of Ring Spot Virus and the A.Y. Strain*— The virus of Ring Spot disease of tobacco does not readily infect the tomato, which can only be infected with this disease by grafting (Price, 1933). The virus is easily transmitted by rubbing to tobacco, where it induces the development of the chlorotic rings which give the disease its name, with the subsequent development of some necrosis (Price, 1933). A series of tobacco plants were inoculated with Ring Spot virus kindly given to me by Dr. J. M. Birkeland. After the symptoms had become well marked on the inoculated leaves, the leaves were rubbed with juice containing A.Y. virus. In 3 days marked necrotic lesions appeared on the rubbed leaves and there was not the slightest evidence that the presence of Ring Spot virus affected the development of the A.Y. strain except in so far as the reduction of growth in the infected plants tended to make the symptoms induced by the A.Y. virus less marked than they would have been in normal infected plants. Apart from that, the Ring Spot virus appears to have no specific effect on the virus of Yellow Mosaic. The presence of active A.Y. virus was demonstrated by inoculating some of the upper leaves of the tobaccos into young tomato plants into which Ring Spot virus cannot be introduced by juice inoculation. In these plants, typical symptoms of Yellow Mosaic readily developed.

Discussion

A considerable volume of work on plant virus diseases and on the nature of the causative agent has established the following facts:—

(*a*) There exist virus complexes, which, especially in the potato diseases, can be separated more or less readily into their component viruses. This may be effected by passage through " filter plants " by heat treatment or by making use of some other differences in the host and *in vitro* reactions of the viruses. Different mixtures of these viruses may give rise to different disease symptoms in the appropriate host plants.

(*b*) The virulence of a virus may sometimes be increased or diminished by passage through an unusual host or by special treatment *in vitro*. Passage through *Chenopodium murale*, for example, attenuates the virus of Curly-Top disease of sugar-beet, while subsequent passage through *Stellaria media* restores its virulence. Johnson (1928), Kunkel (1934), and others have shown that the exposure of tobacco plants to a temperature of 35°–36° C for some days after inoculation not only seriously affects the expression of the symptoms produced, but also attenuates the viruses of the tobacco Mosaics since these viruses are recovered in a much weakened form.

The separation of mixtures of viruses into their components and the alteration in virulence of any given virus must not be confused with the isolation of separate strains of the same virus.

Hitherto there has been little evidence whether or not a particular virus might occur in different strains. McKinney (1926) has reported that the small yellow spots which appear on tobacco after infection with tobacco Mosaic give rise on isolation to a Yellow Mosaic as against the green type from which the original inoculation was made. The green symptoms, he found, could suppress the yellow, and if the virus which caused the yellow symptoms were in high dilution the yellow spots did not appear. It is not clear whether this is a case of segregation of two viruses or of separation of two strains of the same virus. Jensen (1933) has recently reported a similar group of experiments with tobacco Mosaic virus (*T.V. No.* 1). Bennett (1932) in a recent paper suggests that the different Mosaic diseases of raspberry may be due to strains of the same virus which have different degrees of virulence. Cooley (1932) has found, also in raspberry, that there are two Streak diseases, which, differing in one symptom only, are similar in others and appear to be closely related. Storey and McClean (1930) described strains of different virulence of the virus of " Streak " disease of maize. More recently Salaman (1933) has published evidence of the existence of at least two strains in the X virus of potato Mosaic which causes a distinct Mosaic in tobacco. He found that there were apparently different strains in the yellow and in the green areas of the infected tobacco leaf, and that the " green " strain induced immunity against the " yellow," if inoculated first into tobacco or *Datura stramonium*.

The general papers of Chester (1933) summarize the present position with regard to immunity in plants. A considerable portion of these papers is devoted to a consideration of immunity to virus diseases and a comprehensive survey of the available literature is made.

While this paper was in course of preparation Kunkel (1934) published

a paper in which he describes some experiments with strains of Yellow Mosaic virus (from the stock kept in this laboratory), these strains being isolated and studied in *Nicotiana sylvestris* plants. He considered one of the strains an attenuated form of the other, and found that the attenuated virus immunized plants against the virulent form, or against ordinary tobacco Mosaic virus (*T.V. No.* 1). The second virus did not appear to multiply in the protected tissues. Cucumber Mosaic virus or tobacco Ring Spot virus did not induce immunity against the Yellow Mosaic virus.

The author's experiments establish the fact that the virus causing Yellow or Aucuba Mosaic of the tomato does exist in at least two strains, which cannot by any known means be changed, the one into the other. Evidence has been adduced to show that the one cannot be considered as an attenuated form of the other, as no return of virulence has been induced by frequent passage through susceptible host-plants.

The experiments further show that inoculation of the plant with one of these strains prevents the multiplication of the other strain in the tissues of the infected plant, and consequently that complete immunity against one strain is induced by infection with the other.

This observation has been extended to other viruses and it has been found that four types of interaction may be recognized—

(*a*) The first interaction consists of the induction of complete immunity to a second virus by infection with a first. In this type the second virus apparently does not multiply in the host tissues and none of the second virus can be recovered from the inoculated plant.

(*b*) The second type of interaction is found with those viruses, the first of which apparently confers some degree of immunity against the second, the symptoms associated with the second virus not developing in the inoculated plant, or the development of the second virus being very much reduced by the presence of the first.

(*c*) The presence of a virus in the tissues of a plant may actually increase the severity of the symptoms induced by a second virus, making the disease caused by the two viruses more severe than that caused by either virus alone.

(*d*) The presence of a virus in the tissues of a plant may interfere with the development of a second virus insofar as the reduction in the growth of the host plant reduces symptom expression. The two viruses seem to multiply together in the tissues without mutual interference.

Some general considerations arise from this discussion. The presence of strains in plant viruses is probably much more general than was previously believed and it may be that some of the present anomalies in

symptom expression may be due to the unsuspected presence of strains, one of which induces one type of symptom, the other another. These may occur in differing proportions under certain environmental conditions. Certainly, the different effect of summer and winter conditions is much less marked with plants infected only with the A.G. strain or the A.Y. strain of *T.V. No.* 6 than it was with the original Yellow Mosaic virus, which was presumably a mixture.

The problem of the immunity against one strain of virus induced in plants by an infection with another strain is of great interest from the point of view of the light its solution would throw on the nature of the virus. The amount of virus in any given tissues must be comparatively small, having regard to the size of the virus particle. The virus of Yellow Mosaic, with which this paper largely deals, has a diameter of approximately 40 $\mu\mu$ (McClement and Smith, 1932) and this refers to the size of the virus unit necessary to induce a necrotic lesion on the leaves of *N. glutinosa*—not necessarily to the size of a single virus body.

The number of these units per cubic centimetre in any given juice has been shown by the author (Caldwell, 1933) to be comparatively small. There must be, therefore, either only a few foci in the tissues at which the virus can multiply, or some reaction of the plant to the virus infection which prevents the multiplication of a second virus sensitive to the presence of the first. The second explanation seems the more probable since the multiplication of two mutually tolerant viruses in a tissue could only be accounted for, on the first hypothesis, by the assumption that different viruses had different foci of development in the plant. If two viruses are mutually exclusive, the second hypothesis would suppose the first virus to have "saturated" all the available foci, preventing thereby the multiplication of the other.

Summary

Two strains of the virus of Yellow Mosaic of tomato (Johnson's *T.V. No.* 6) have been isolated and their reactions determined. Evidence is adduced to show that one virus is not an attenuated form of the other, and that many of the anomalies now observed in symptom expression in host plants may probably be referred to the existence of strains not hitherto recognized. It has been found that one strain apparently completely immunizes the host plants against the other. This observation has been extended and it has been found that four types of interaction between viruses can be distinguished.

(*a*) A virus may completely inhibit the development of another in the host tissues.

(*b*) The second virus may multiply in the tissues without inducing typical disease symptoms.

(*c*) The two viruses may multiply and induce symptoms each inducing the typical symptoms of its specific disease.

(*d*) The effect of the second virus may be to cause a more severe disease than either virus could of itself have caused.

The significance of these observations in relation to the multiplication of the virus in the tissues of a host plant is discussed.

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Description of Plates

Plate 14

Fig. 1—Tomato plant infected with virus of the A.Y. strain.
Fig. 2—Similar tomato plant infected with virus of the A.G. strain.

Plate 15

Fig. 3—Hair of leaf of *S. nodiflorum* showing inclusion bodies typical of *T.V. No.* 6.
Fig. 4—Hair of leaf of *S. nodiflorum* showing inclusion bodies of mixture of *Tom. S.V. No.* 1 and *T.V. No.* 1.
Fig. 5—Leaf of *Zinnia sp.* infected with A.Y. strain.
Fig. 6—Leaf of *N. glauca* infected with A.Y. strain.

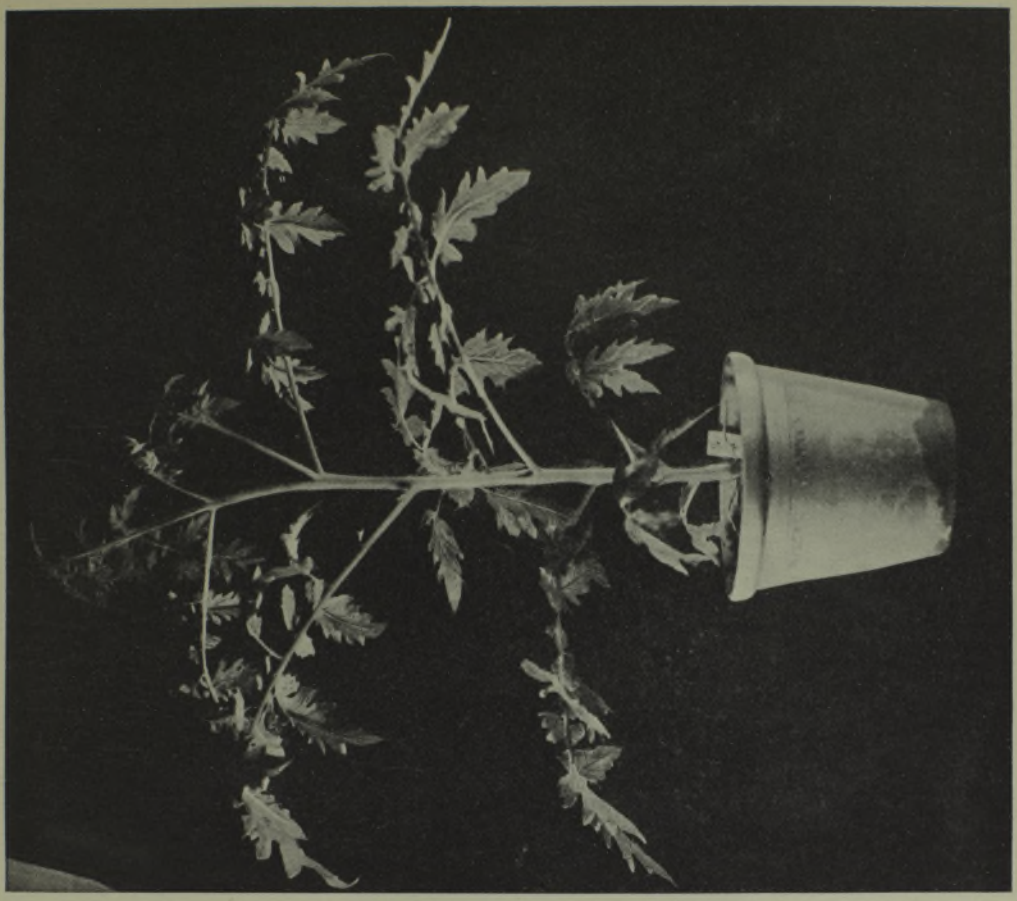

FIG. 2

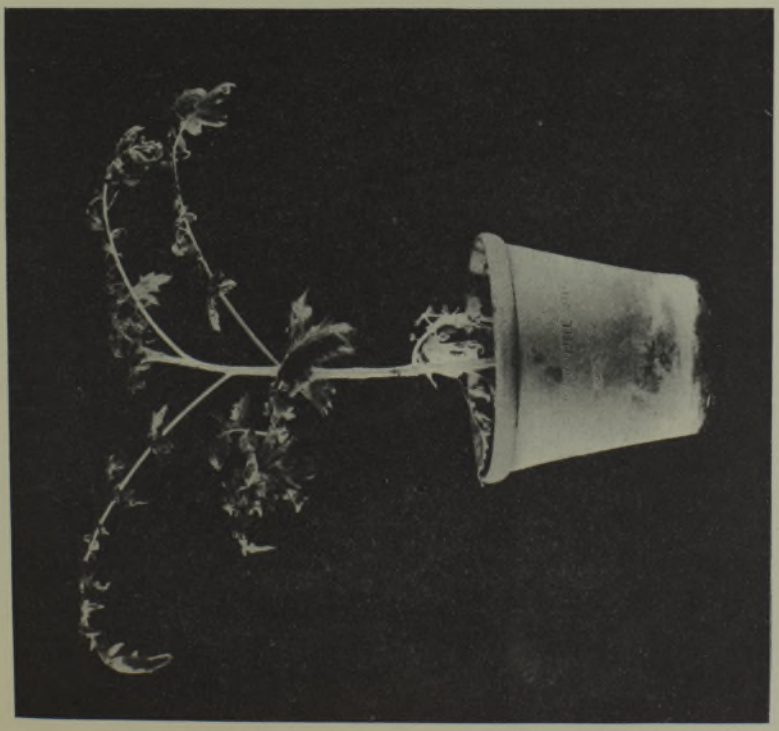

FIG. 1

(Facing p. 138.)

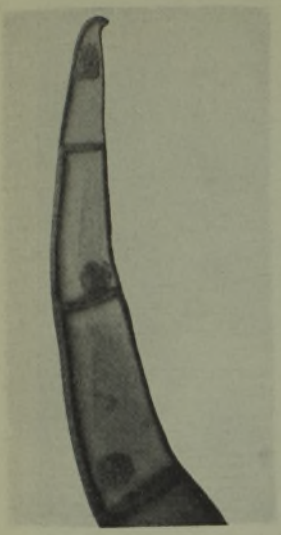

FIG. 3

FIG. 4

FIG. 5

FIG. 6

FIG. 7

FIG. 8

FIG. 9

FIG. 10

PLATE 16

FIG. 7—Leaf of tomato plant infected with A.Y. strain.
FIG. 8—Leaf of tomato plant infected with A.G. strain (note almost complete absence of symptoms).
FIG. 9—Leaf of tomato plant infected with "X" virus and later with A.Y. strain.
FIG. 10—Leaf of tomato plant infected with *Tom. S.V. No.* 1 and later with A.Y. strain.

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The Dissolved Constituents of Human Sweat

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In this paper the object has been to obtain data as to the dissolved constituents of sweat collected from the human body, and at the same time to endeavour to distinguish what is secreted by the sweat-glands from what is derived from the general surface of the skin.

The experiments were carried out, as previously, in connection with the experimental chamber of the Mining Research Laboratory, University of Birmingham, and mainly during the author's tenure of the Tyndall Studentship. One of the subjects (A. H. H.) was a medical student of good physique who was known to sweat much more freely than the average individual.

METHODS OF ANALYSIS

Most of the methods used in analysing the sweat washings have been described previously (Hancock, Whitehouse, and Haldane, 1929) and it will only be necessary, therefore, to deal with the determinations of lactic acid and urea.

Lactic Acid—The method described by Friedemann, Cotonio, and Shaffer (1927) was employed, using the modified condenser unit of Davenport and Cotonio (1927). The determination consists briefly in oxidizing the lactic acid to acetaldehyde by acid permanganate in presence of manganous sulphate. The acetaldehyde formed is aerated out of solution, absorbed in sodium bisulphite solution, and determined by titration. Immediately after collection the sweat washings were treated for the removal of sugar and other interfering substances by the copper-