
METHUEN'S
MM
BIOLOGICAL MONOGRAPHS

METHUEN'S
MONOGRAPHS ON
BIOLOGICAL SUBJECTS

General Editor: KENNETH MELLANBY

PLANT VIRUSES

Plant Viruses

KENNETH M. SMITH
C.B.E., D.Sc., Ph.D., F.R.S.
Agricultural Research Council
Virus Research Unit, Cambridge

LONDON: METHUEN & CO LTD
NEW YORK: JOHN WILEY & SONS, INC

First published October 3, 1935
Second edition 1948
Third edition, revised and reset, 1960
Printed and bound in Great Britain
by Butler & Tanner Ltd., Frome
3.1
CATALOGUE NO. 2/4112/11

Preface to the Third Edition

The aim of this small book is to make it possible for a student, without previous experience, to gain some idea of the basic principles of plant virology and the means by which our knowledge has been obtained.

The first part of the book deals briefly with the virus in the plant, the diseases caused and their physiology, movement in the plant and methods of dissemination. Next the virus in the arthropod vector is discussed, and lastly the virus itself is examined in the electron microscope.

With the increasing importance of virology and the growing recognition of the subject as an independent science, a need is felt, particularly in plant virology, for some account of practical methods of study. An attempt has therefore been made in the second half of this book to bring together all the various techniques used in the study of plant viruses which have been developed over the last two or three decades.

It is obviously not possible in a book of this size to give detailed accounts of the more specialized and complicated techniques such, for example, as those necessary in the electron microscopy of viruses, and for these the reader is referred to the literature of the subject. Nevertheless, it is hoped that the book will be of help to the student who is beginning the rather difficult but fascinating study of plant viruses.

Grateful acknowledgement is due to Miss M. E. Short for her assistance in finding many of the references, to Mr. G. J. Hills for making the electron micrographs, and to Mr. Simon Frey for taking the photographs for Plates I–III and VII.

Acknowledgement is also due to the Society of General

Microbiology and the Cambridge University Press for permission to reproduce the article on the relationship of plant viruses with their arthropod vectors from the seventh symposium of the Society on Microbial Ecology.

KENNETH M. SMITH

Agricultural Research Council
Virus Research Unit,
Huntingdon Road, Cambridge

Contents

PART II

PRACTICAL METHODS IN PLANT VIROLOGY

Plates

PART I

GENERAL SURVEY

CHAPTER I

Introductory

HISTORICAL

Virus diseases of plants, although not of course recognized as such, were known long before the discovery of bacteria. The first record in the literature of which we have knowledge is a description published in 1576 by Charles l'Ecluse or Carolus Clusius of a variegation in the colour of tulips, which is now called 'breaking' and is recognized to be due to an aphid-transmitted virus of the mosaic type. 'Broken' tulips are figured in *Theatrum Florae*, published in 1662; these illustrations have been identified as the work of the painter Daniel Rabel. A somewhat later account published in *Traité des Tulips* about 1670 contains the first suggestion that the variegation in the flower colour might be due to a disease. In 1715 an account of an infectious chlorosis of *Jasminum* was published in the *Art of Gardening*.

About fifty years later the so-called 'curl' disease of potatoes came into prominence, and about the cause of this there raged for many years a great controversy. The favourite explanation was that of 'degeneration', a kind of senile decay caused by long-continued vegetative propagation. It was pointed out, however, that in certain secluded districts, high up on mountains or in wind-blown areas near the sea, it was possible to grow the same variety of potato for many years, saving the 'seed' each year from the current year's crop, without any sign of degeneration. It was the discovery that potato leaf-roll was an infectious

virus disease which finally settled this controversy and showed that the degeneration of the potato crop was due solely to a gradual infiltration of viruses into the stocks.

About 1868 the variegated plant *Abutilon*, probably *A. striatum* var. *Thompsonii*, appeared in Europe and became popular as an ornamental plant. By grafting scions of variegated plants to green shoots of normal plants it was discovered that this variegation was infectious. Now the variegation in *Abutilon* is known to be due to a virus infection transmitted by a species of whitefly.

In 1886 Mayer described a disease of the tobacco plant which he called *Mosaikkrankheit*, and this term, or its English equivalent, is now widely used for describing the mottling type of virus disease. Mayer showed that this mosaic disease of tobacco could be communicated to a healthy tobacco plant by inoculation with the sap of the infected plant. Two years later Erwin F. Smith proved that the disease known as 'peach yellows' was also communicable and could be transmitted by budding.

It was not, however, till 1892 that the first scientific proof of the existence of a virus was given. Iwanowsky (1892) working with the mosaic disease of tobacco, described by Mayer, proved that sap from such a diseased plant was capable of inducing the mosaic disease in healthy tobacco plants *after* it had been passed through a bacteria-proof filter candle and was bacteriologically sterile. Curiously enough, Iwanowsky himself did not seem to grasp the true significance of this and his discovery passed almost unnoticed until the work was repeated seven years later by Beijerinck (1898), who then propounded his theory of a *contagium vivum fluidum.*

The discovery of the relationship between viruses and insects was not made in a day, and a period of years elapsed between the time when insects were first suspected and the actual demonstration of this method of transmission. The first to prove experimentally the relationship between an insect and a plant virus seems to have been a Japanese farmer, Hashimoto, who worked in 1894 with

the dwarf disease of rice and the leafhopper *Nephotettix apicalis* var. *cincticeps*.

About 1907 three workers in America – Ball, Adams, and Shaw – suggested that there was some connexion between curly-top of sugar-beet and the leafhopper *Eutettix tenella*. In 1915 Smith & Boncquet (1915) confirmed this and showed that a single insect from an infected plant placed on a healthy plant for 5 minutes would produce the disease.

In his historical review of plant viruses and virus diseases, Cook (1946) divides the history of virus study into three arbitrary periods. The initial period begins with the first records of a virus disease, that of tulip 'breaking' or mosaic by Carolus Clusius in 1576, and may be said to end in 1868 with a description of the variegation of *Abutilon striatum*. During this period there was no research as that is understood at the present time, but there were some discoveries of importance. These were (1) that the 'breaking' of tulips was transmitted by bulbs from plants showing these characteristics; (2) that peach yellows and the mottlings of *Abutilon striatum* var. *Thompsonii* were transmissible by budding, and (3) that when a mottled branch of *A. striatum* var. *Thompsonii* was grafted into a fresh plant, the mottling appeared in the new green leaves.

The second period may be said to begin with the work of Mayer, who in 1888 made a study of the mosaic disease of tobacco and showed it to be transmissible. This period also includes the work of Iwanowsky and Beijerinck already referred to. Cook puts the beginning of the third period at about 1906 when the study of plant viruses was commencing, but this did not become intensive until at least two decades had passed.

To these may now be added a fourth period beginning with the isolation of tobacco mosaic virus by Stanley in 1935 and the pioneer work of Bawden & Pirie. These advances allow us in the words of Markham (1959) to regard the plant viruses 'as commonplace chemicals used

for calibrating physical apparatus of various kinds and as sources of nucleic acid'.

In the ten years or so since the last edition of this book was published the advances in our knowledge of viruses, including those infecting plants, have been remarkable. Perhaps the greatest emphasis has been laid on the virus nucleic acids and, as the editors of the ninth symposium of the Society of General Microbiology put it, 'the central theme is the importance of viral nucleic acids in virus growth and variation, and the methods by which viral nucleic acids impress their stamp on the cell and induce the production of new virus copies'.

There is now much circumstantial evidence that the virus particle, on entering the cell, sheds its protein coat, so that the nucleic acid is liberated before multiplication begins. Workers in Germany and the U.S.A. have reconstituted the tobacco mosaic virus particle from the protein and nucleic acid components and hybrid viruses have been produced. Moreover, the nucleic acid has been shown to be itself infective.

X-ray studies have elucidated the structure of the tobacco mosaic virus particle and some of the smaller plant viruses have been shown to have regular geometric shapes and several seem to be icosahedra.

There have been great improvements in the ultracentrifuge techniques and a new differential centrifugation in a sucrose gradient has been evolved.

New developments in the technique of electron microscopy include the cutting of ultrathin sections and the use of electron stains which allow the protein sub-units of the virus particle to be directly visualized.

More knowledge has been obtained on the relationship of plant viruses with their vectors and on the methods of transmission. Besides much new evidence on the multiplication of some plant viruses in their insect vectors, there is a suggestion that there may be a deleterious effect of such multiplication on the insects themselves. Several leafhopper transmitted viruses have been discovered in western

Europe and more light has been thrown on the relationship between plant viruses and the aphid vectors. Mites have been incriminated as the vectors of several viruses and the first instance of a nematode vector of a plant virus has been recorded.

Contrary to long-established belief that soil-transmitted viruses were rare, a number of such viruses, some of considerable economic importance, have been discovered.

REFERENCES

BEIJERINCK, M. W. (1898). 'Ueber ein contagium vivum fluidum als ursache der fleckenkrankheit der tabaksblatter.' *Verhandel K. Akad. Wetensch. Amsterdam*, Sec. 2, Deel 6, 1–22.

COOK, M. T. (1946). 'Plant Viruses and Virus Diseases.' Mimeo. Dept. Bot., Louisiana State Univ.

L'ECLUSE, C. DE (1576). *Rariorum Aliquot Stirpium per Hispanias Observatarum Historia.* Pp. 529 illus. Antwerpiae.

IWANOWSKY, D. (1892). 'Ueber die Mosaikkrankheit der Tabakspflanze.' *St. Petersb. Acad. Imp. Sci. Bull.* **35**, 67–70.

MARKHAM, R. (1959). 'The biochemistry of plant viruses.' *The Viruses.* 2. Eds. F. M. Burnet and W. M. Stanley. New York: Academic Press.

MAYER, A. E. (1882). 'Over de mosaikziekte van de tabac.' *Woorloopie Meded. Landbou. Tidschr.* 359–64.

SMITH, R. E. and BONCQUET, P. A. (1915). 'New light on curly top of sugar beet.' *Phytopathology*, **5**, 103–7.

CHAPTER II

Symptomatology

EXTERNAL SYMPTOMS

Primary Symptoms

The initial or primary symptoms of a plant virus disease may differ substantially from those which develop from the systemic spread of the virus throughout the plant. There are two main types of primary symptoms and they relate chiefly to viruses which are mechanically transmissible. The first is a local reaction at the actual site of inoculation and this consists of spots or rings of various types; they are usually necrotic but are occasionally chlorotic. They are known as 'local lesions' and, because they have a practical application in the quantitative study of viruses, they are dealt with in some detail in a later section of the book.

The second type of primary symptom is known as 'clearing of the veins', a condition where the veins of the youngest leaves become conspicuous by reason of a yellowing or necrosis; this is common in many of the mosaic diseases. Such a condition is transitory and disappears as the systemic disease develops.

The rate of development of local lesions is dependent upon a number of factors such as environmental conditions before and after inoculation, whilst the species of host plant inoculated governs to a large extent whether lesions shall or shall not develop. For example, tobacco mosaic virus (TMV) does not, with certain exceptions, give rise to local symptoms on the inoculated leaves of the tobacco plant. The primary symptom in this case is the

appearance, after some days, of clearing of the veins of the youngest leaves. This is followed after an interval by the development of the characteristic systemic disease. On the other hand, inoculation of *Nicotiana glutinosa* with TMV invariably produces numerous necrotic spots on the inoculated leaves. Except at high temperatures, no further development of the disease in *N. glutinosa* takes place. In the case of certain strains of TMV, notably that causing mosaic of tomatoes in commercial glasshouses, local lesions without further spread of the virus occurs in tobacco as well as in *N. glutinosa*. In some varieties of tobacco, however, e.g. Kawala, the virus of tomato mosaic may become systemic.

The appearance of local lesions is the most rapid development of virus symptoms; the period may be as short as 36–48 hours as compared with 5 to 10 days for clearing of the veins.

Many of the sap-transmissible mosaic-type viruses give local lesions, though not on all plant hosts, and the search for a local lesion host is an important factor in the study of a newly discovered virus.

Occasionally there may be primary and secondary phases in a virus disease which is not mechanically transmissible. In potato leaf-roll, for example, the rolling and pallor are confined to the young leaves in the primary condition; but in the secondary phase the lower leaves are also affected, being crisp and dry.

Systemic Symptoms

In considering systemic virus diseases in plants it is important to remember that the symptomatology of a given disease is not an unchangeable picture which is always reproducible in a certain plant by a certain virus. On the contrary, there are many variable factors governing the development of symptoms so that the over-all picture is never quite the same, though there may be one distinguishing feature which recurs with fair regularity.

Of these variable factors the following are the most

important, first the virus itself which may exist in a number of strains, differing in virulence. For example, one strain of TMV may cause a bright yellow mottling on the leaves, a second may cause necrosis only, whilst a third induces a gross malformation; all these symptoms differ substantially from the green mottle characteristic of the type strain of TMV. Another variable factor is the variety of plant affected, the varietal difference in response to the same virus being very marked in some cases, particularly with the potato plant and potato virus X. One potato variety may react to this virus with necrosis of the top part of the plant, another with a mottling, whilst quite a number will carry the virus without symptoms. This extreme variability in reaction was the cause of much confusion in the early days of potato virus research. Light and temperature also play a considerable part in affecting the symptomatology of plant virus diseases, and this is discussed again later in this chapter.

We can now describe briefly some of the main expressions of systemic virus diseases.

Mosaic Mottling. The word mosaic was first used by Mayer (1882) to describe the symptoms caused by TMV in the tobacco plant, owing to a fancied resemblance to a mosaic pattern. The word is now used to describe those diseases, caused by a multiplicity of viruses, in which the leaves show a mottling of light or dark green, yellow or even white. 'Banding' of the veins with a dark green is another form of mottling. Associated with the mottling is often found a greater or less degree of alteration of leaf symmetry, crinkling of the edges, blistering, and so on. Sometimes the leaf blade is reduced so that numbers of filiform leaves are produced.

Ringspotting. Associated with the mosaic type of symptoms is another manifestation in which numerous rings, usually concentric with a central spot, develop on the leaves. With these 'ringspot viruses', so called, the primary symptoms are usually also in the form of rings. As the disease progresses the rings are frequently replaced by the develop-

Fig. 2. Tobacco mosaic virus on tobacco; ordinary or field strain

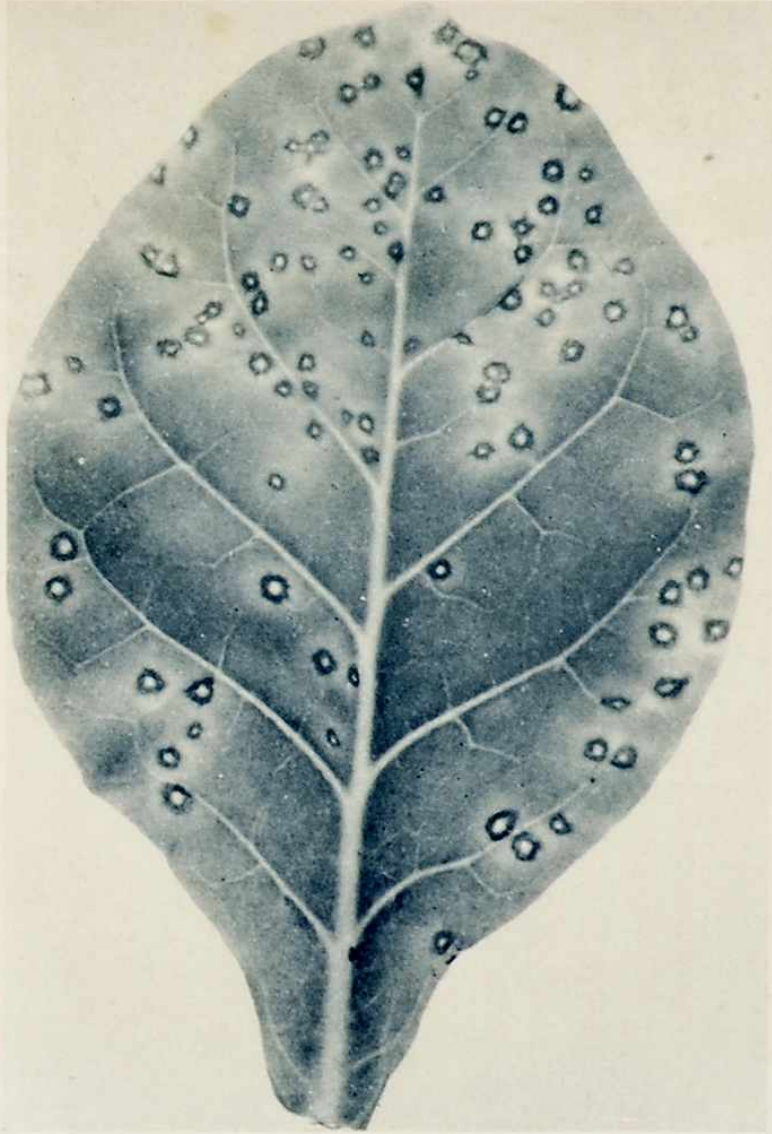


Fig. 1. Cabbage black ringspot virus on tobacco; an example of local lesions

Plate I

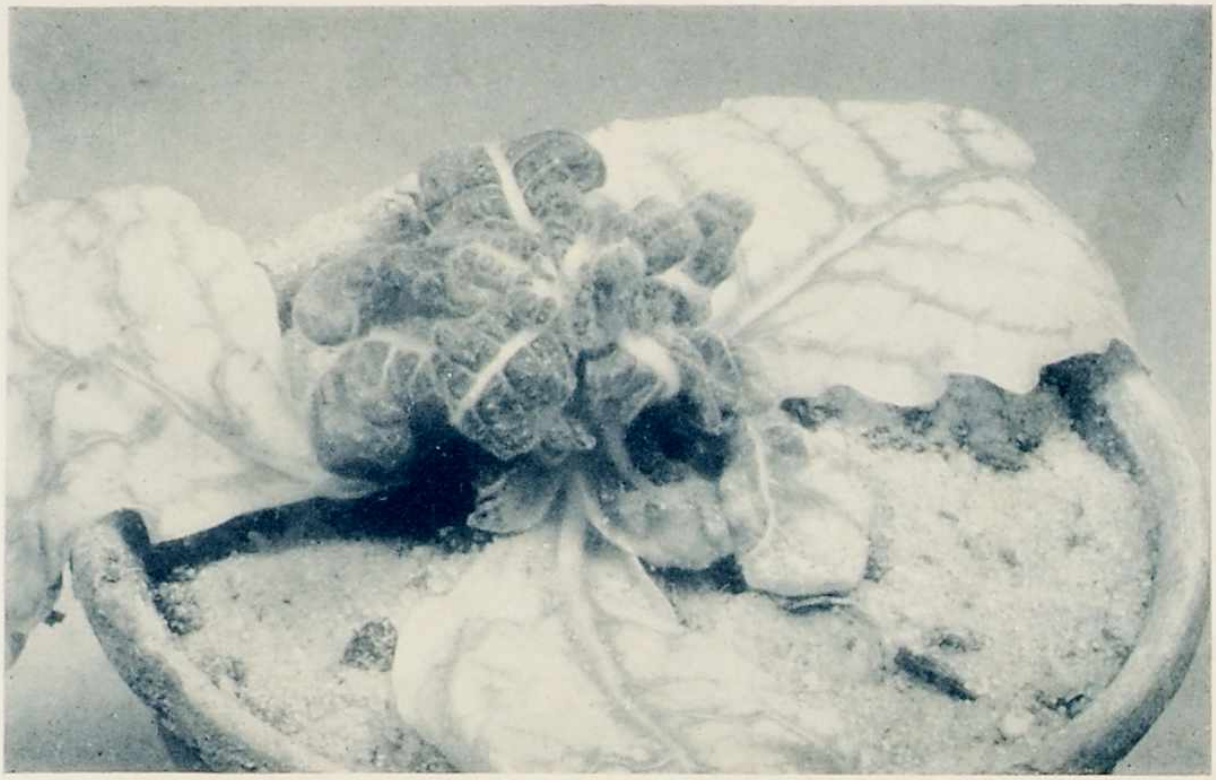


Fig. 3. Tobacco rosette, an example of a composite virus disease

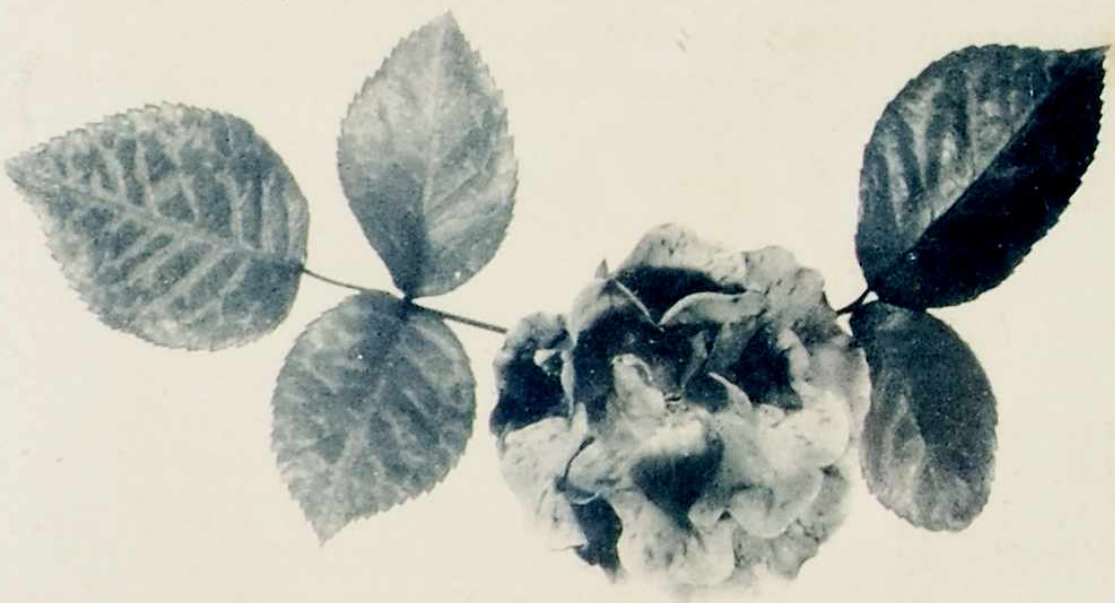


Fig. 4. Veinal mosaic and colour break on Floribunda rose, Else Poulsen

ment of lines forming an 'oakleaf pattern', and this in turn may give place to an ordinary mosaic mottle.

Chlorosis. Uniform yellowing, or general chlorosis, without patterns of different shades of green is not common in plant virus diseases. It occurs to a certain extent in the group of disorders, known as 'yellows' and dealt with in a subsequent paragraph, but here there are many other more outstanding symptoms. Perhaps the best example of chlorosis alone is given by a disease of sugar-beet, known as 'virus yellows', in which the main symptom is a uniform yellowing of the leaves.

Distortion and Outgrowths. Distortion of the leaves is a common symptom of virus disease and may take the form of crinkling, rosetting, or loss of the lamina altogether. The cucumber mosaic virus, and its strains, is particularly liable to produce distortions and strap-like leaves in some hosts, notably *Nicotiana glutinosa*; one strain of this virus gives rise to secondary leaves which grow out of the midribs of other leaves. A less extreme type of outgrowth known as 'enations' is caused in one or two hosts by TMV, by the tobacco rosette complex, by tobacco leaf-curl virus and by tomato black-ring virus on hot-house cucumber leaves. These enations, which develop on the undersides of leaves, vary from a few millimetres in depth to a centimetre or more.

Necrosis. Death of the cells, or necrosis, is a symptom of many virus diseases, and may consist of small areas on the leaves, streaks on the stem or large areas of dead tissue which ultimately cause the death of the whole plant. As we have already shown, many viruses produce necrotic local lesions, and these are sometimes followed by a systemic necrosis. A good example of this is given by a strain of TMV which produces, under certain conditions, a necrotic streak or stripe on the stems of tomato plants together with necrosis of the leaves and fruit. There is one group, the tobacco necrosis viruses, which give rise only to necrotic symptoms on leaf and stem.

Yellows. This name has been given to a group of diseases

having a number of characteristics in common. In the writer's opinion the word is poorly descriptive of the symptoms, since yellowing is by no means outstanding. The following are some of the main symptoms which occur in aster yellows as described by Kunkel (1926). Clearing of the veins is typical of early infection, followed by chlorosis of the leaves, the production of secondary shoots and an upright habit of growth. When the attack is severe the young leaves are almost white; while yellows depresses chlorophyll-production in portions of the plant normally green, it causes the production of a green-coloured substance in the floral parts where chlorophyll is not normally present. The flower heads are always more or less dwarfed. Individual flowers in some cases develop into vegetative branches which may or may not bear small flower heads. Trichomes on diseased flowers frequently develop into leaflike structures. Diseased leaves are somewhat deformed with petioles longer than those of healthy leaves of the same age. The leaf blade is narrower, and on the whole smaller than the normal blade. Diseased leaves may have deep clefts and notched margins, but are seldom severely deformed. Necrosis is also a symptom of advanced stages of yellows and is most severe in the stem tissue just below the apical bud of the main stem.

Root Symptoms

As a rule the roots of affected plants do not show very marked symptoms, but changes do sometimes occur as, for example, in maize stunt disease, where the roots are stunted and excessively branched (Kunkel, 1948). In the wound-tumour disease of clover and other leguminous plants, large numbers of tumours tend to form on the roots. As the name implies, tumours tend to form at the site of wounds, and the breaking of numerous root hairs during the normal process of growth is probably the reason for the formation of many tumours on the roots.

Fruit and Flower Symptoms

Many viruses give rise to symptoms on the fruit. In the

tomato plant, the virus of tomato spotted wilt frequently produces rings on the ripe fruit; other examples are chlorotic spots or ring-like blotches due to tomato bushy-stunt virus and necrosis caused by tomato streak virus.

In cucumber mosaic the stem-end of the young cucumber becomes mottled with yellowish-green, and this gradually spreads over the entire fruit. There are frequently portions of a dark green usually raised above the surrounding surface in such a way as to produce wart-like projections.

There are numerous examples of stone fruits affected by virus diseases; peaches with the red suture disease may have a rough or bumpy contour and show an abnormal deep red to purple blush over the exposed side. In peach yellows the fruit ripens prematurely; the skin of the peach is highly coloured, spotted with red or purple and the flesh marbled with crimson with pronounced colouring round the pit. The pear stony-pit virus causes deformed areas in the fruit which become necrotic or corky, but the most striking feature of the diseased pear is the production of numerous schlerenchyma cells beneath or surrounding the pitted areas. These fruits become so gnarled or woody that they are difficult to cut with a knife.

Sometimes the chief symptom is a reduction in the size of the fruit as in the 'little-cherry' disease which is caused by the virus of peach X-disease. Here the fruits on affected trees are about half the size of those on healthy trees, and in some cherry varieties may be more angular and pointed than normal.

Symptoms also occur in fruits other than stone fruits; for example, in apple rough-skin disease, as the name implies, rough brown patches, stripes and, in some cases, rings develop on the fruit. A somewhat similar state of affairs develops in the apple ringspot virus disease.

A characteristic flower symptom caused by virus infection is a colour change, usually referred to as a 'colour break'. The best known of this type of symptom is the tulip break which is due to a segregation of the anthocyanin pigment in the epidermis of the petal as fine featherings

about the margin or in irregular stripes up the middle of each segment, while between the stripes or streaks appear patches of more or less clear ground colour, usually white or yellow. Another common flower break occurs in the blood-red wallflower (*Cheiranthus cheiri* L.) in which the red flowers become striped or flecked with a yellow variegation. The pink flowers of gladioli often show a white streak, and this is usually due to infection with cucumber mosaic virus. On the whole, it is the mosaic type of disease which tends to induce flower breaks.

One of the most striking alterations produced in the flowers of an affected plant is that due to cranberry false-blossom disease, one of the 'yellows' type previously referred to. The flowers assume an upright position instead of the normal curve of the pedicel. The calyx lobes of the diseased flowers become enlarged, the petals are short and streaked with red and green and the stamens and pistils are more or less abnormal. When the disease is severe the entire flower may be replaced by successive whorls of leaves or by a short branch.

INTERNAL SYMPTOMS

Intracellular Inclusions

Under this heading are dealt with inclusions which develop in the cell cytoplasm; intranuclear inclusions are briefly considered in an ensuing paragraph. Iwanowsky (1903) was probably the first to point out that certain abnormal intracellular inclusions were always present in some cells of mosaic-infected tobacco plants. These abnormal inclusions are characteristic of virus infections, and whilst they are not present in all virus diseases, they do not occur in diseases caused by other infectious agents. After Iwanowsky's discovery, Matz (1919) found plasmodium-like masses in the cells of mosaic-infected sugar cane and in 1921 Kunkel described intracellular inclusions, similar to those described by Iwanowsky, in maize affected with a mosaic disease. Not long after this Smith (1924) reported

the occurrence of amoeboid bodies in the cells of potato plants infected with mild mosaic (potato virus X).

There are several different kinds of intracellular inclusions and they can be roughly classified into (*a*) crystalline or fibrous, (*b*) amorphous, known as X-bodies, (*c*) intranuclear inclusions, (*d*) other types of inclusions.

The crystalline inclusions occur mainly in the cells of plants infected with tobacco mosaic virus and are usually in the form of plates of varying size. Iwanowsky showed that the plates develop striations and seem to be composed of needle-like crystals when acidified.

There is considerable variation in the shape and form of these crystalline inclusions and the type of inclusion seems to be determined in a slight degree by the host plant but is largely controlled by the amount of light and heat available to the host (Kassanis & Sheffield, 1941).

In addition to the tobacco mosaic virus and its strains one or two other viruses give rise to crystalline intracellular inclusions. McWhorter (1941) has described isometric crystals in the cells of leguminous plants affected with the viruses of pea mosaic and yellow bean mosaic. Very fine protein crystals have also been observed in the cells of virus-infected cacti, *Rhipsalis cerenscula* (Weber, Kenda & Thaler, 1952).

Amorphous Inclusions or X-bodies. There is a good deal of evidence which suggests that the amorphous inclusions are very similar to the crystalline inclusions because ultimately the body breaks down to give a number of protein crystals. There are many references in the literature to these X-bodies and their morphology, and only a few can be dealt with here.

The formation and disappearance of the X-bodies in the tobacco mosaic disease, known as aucuba mosaic, has been examined in detail by Sheffield (1931), who made a cinematograph film of X-body formation. According to her observations the rate of streaming of the cytoplasm increases soon after infection and minute particles of protein appear which are carried about by the cytoplasm of the

cell. These particles aggregrate and fuse to form larger masses of which there may be several in one cell. The intracellular inclusions caused by three strains of tobacco mosaic virus have been studied in some detail. The amorphous bodies of all three diseases are relatively stable and are preserved by ordinary cytological fixatives. In the case of tobacco mosaic and enation mosaic the bodies average about 10 μ in length and contain vacuoles, chondriosomes and oil globules (Bawden & Sheffield, 1939).

In addition to the tobacco mosaic viruses, large numbers of amorphous inclusions occur in all tissues of plants infected with *Hyosyamus* mosaic virus, and tobacco ringspot virus also causes the production of many amorphous inclusions (Bawden & Sheffield, 1939).

Intracellular inclusions occur also in the cells of plants infected with cabbage black ringspot, cauliflower mosaic and turnip yellow mosaic (Rubio, 1950). Similar inclusions have been observed in the cells of broad bean plants (*Vicia faba*) infected with the virus causing broad bean mottle (Rubio & van Slogteren, 1956).

Intranuclear Inclusions

Unlike the virus diseases of insects in which the nucleus is frequently the site of virus multiplication and consequent inclusions, intranuclear inclusions are rare in plant virus diseases.

Kassanis (1939) has described such an inclusion in the nuclei of solanaceous plants affected with the virus of severe etch. The inclusions are produced readily in both young and old plants and appear to be limited to those parts of the leaves with external symptoms. The bodies consist of thin rectangular plates and usually several occur in each nucleus.

Isometric crystals have been observed in the nuclei of leguminous plants affected with the viruses of pea mosaic and yellow bean mosaic. They occur regularly within the nucleolus and there may be five or more at a time (McWhorter, 1941). Lastly, a yellow-mottling strain of TMV

gives rise to fibrous and crystalline inclusions in the nuclei of infected cells (Woods & Eck, 1948). These workers suggest that this particular strain of TMV is capable of invading the nuclei of cells.

Other Types of Inclusions

There are references in the literature to quite a number of miscellaneous inclusions which occur in the cell cytoplasm of virus-infected plants.

In the root tumour cells of sorrel, *Rumex acetosa* L., infected with the wound tumour virus, a spherical body, called a 'spherule', has been described by Littau & Black (1952). The spherules are hyaline and homogeneous and several may occur in one cell.

There are several instances of spindle-shaped bodies in the cells of plants apparently associated with virus infection. They have been observed in the cells of *Epiphyllum* and *Pereskia* varieties, and could apparently be transferrred to spindle-free plants by grafting (Rosenzopf, 1951).

Numerous fusiform and variably shaped protein bodies have been found in the cytoplasm of the epidermal cells of leaves of *Opuntia brasiliensis*. Inoculation of a spindle-free plant of *Epiphyllum truncatum* with sap from *O. brasiliensis* containing the cytoplasm protein spindles resulted in the development of the spindles in *E. truncatum* within about 3 weeks (Miličič & Plavšić, 1956).

Similar protein crystalloids and so-called 'zebra-spindles' occur in the epidermal cells of the bulb scales of *Lilium tigrinum* and are thought to be of virus origin (Thaler, 1956).

For a long time the nature of the crystalline and amorphous inclusions was obscure, and in the early days of plant virus research there was much controversy on the question. Some workers considered the inclusions to be the aetiological agent and were some kind of organism. In one case the 'organism' was actually classified as a protozoon. Other workers held the view that the inclusions were a pathological reaction on the part of the cell and so were an

effect, rather than the cause, of the disease. In the light of modern research it is interesting to reflect that those who considered the bodies to be the causal agent were in the event nearer to the truth. In 1937 Beale made the important suggestion, which was later to be confirmed by electron microscopy, that the crystalline inclusions of TMV were identical with the needle-like crystals obtained by Stanley (1935) in his original purification of this virus. Beale showed that the crystalline plates transformed into needle crystals upon the addition of dilute acid or salt to the water in which epidermal strips had been mounted under the microscope.

Attempts had been made to isolate the crystalline inclusions of TMV, but these had failed owing to the disintegration of the inclusions at the touch of the microneedle (Sheffield, 1939). By means of a freeze-drying technique this difficulty was later overcome and the crystalline inclusions were removed intact from the cell. Examination under the electron microscope revealed that the inclusions consisted of nothing but particles of TMV and a volatile solvent (Steere & Williams, 1953). Similar studies with the electron microscope of the amorphous inclusions or X-bodies do not give quite such a clear-cut answer as was obtained with the crystalline inclusions, but nevertheless suggest in some cases, at all events, that these also are mainly virus. For example, the X-bodies from the broad bean mottle disease were found to be composed mainly of spherical particles apparently identical with the particles of the purified virus (Rubio & van Slogteren, 1956). Similarly Rubio (1950, 1956) showed that X-bodies of cabbage black ringspot and henbane mosaic consisted of rod-shaped particles similar to those of the viruses concerned. On the other hand, he failed to find any virus particles in the X-bodies of cauliflower mosaic. The whole subject of virus inclusions in plant cells has been recenlyl reviewed by Smith (1958).

EFFECT OF ENVIRONMENT ON THE DEVELOPMENT OF SYMPTOMS

The symptom picture in a plant virus disease is governed by a number of variable factors. These may be summarized as temperature, light, nutrition, virus concerned and strain of virus concerned, host species, the age of the host and the condition of the host in so far as it must be free of a latent virus infection.

A common reaction to high temperature is a total or partial suppression of the symptoms; this is known as 'heat masking' and is often found in some of the mosaic diseases. On the other hand, very low temperatures may have a similar effect, as, for example, with tobacco mosaic (Grainger, 1936). There are some exceptions to heat masking, a striking one being the disease of potato yellow dwarf. Walker & Larson (1939) state that below 16° C. the infected plants may be symptomless but at higher temperatures severe symptoms develop, and at high soil temperatures infected tubers fail to sprout although they may grow normally at lower temperatures.

Pound & Walker (1945a) have studied the effect of temperature on the reactions of certain strains of cabbage black ringspot and cauliflower mosaic viruses. In the first group, the ringspot virus and a strain called cabbage virus A, it was found that the progress and severity of disease development varied directly with the air temperature to which the plants were exposed, symptoms being most severe at 28° C. and mildest at 16°. Marked differences, however, between the reactions of the two viruses to temperature were observed. Symptoms of virus A were distinctly more severe than those of the ringspot virus at 28° C. and 24°, but at 20° and 16° the exact reverse was true. In the cauliflower mosaic group containing the type virus and a strain called cabbage virus B, symptom intensity was also found to be directly proportional to air temperature. However, in contradistinction to the cabbage black ringspot viruses, symptom intensity increased with decrease

in temperature and complete masking occurred at 28° and 24°.

At high temperatures cabbage virus A and the type ringspot virus are indistinguishable on *Nicotiana glutinosa*, but at low temperatures the reactions are very different. Many plant hosts will give a local-lesion reaction to the ringspot virus at low, but not at high, temperatures.

These two viruses also occur in significantly greater concentration in cabbage plants at 28° C. than in cabbage plants at 16°. On the other hand, virus B, a strain of cauliflower mosaic virus, occurs in greater amounts in plants grown at 16° C. than in plants grown at 28° C.

It has been observed in the field that the concentration of cabbage virus A progressively declines as the plants grow into the lower temperatures of the autumn (Pound & Walker, 1945*b*). Progenies of cabbage highly resistant to cabbage black ringspot virus show this resistance under artificial inoculation only at 24° C. or below. At 28° C. infected plants develop severe symptoms. There seems to be a close correlation between symptom severity and virus concentration in the case of the strain known as cabbage virus A. At 16° and 20° C. where resistance was very high, virus concentration was very low, but at 28° where symptoms were severe the virus concentrations were very high (Pound, 1952).

Strains of other plant viruses may also have different temperature relationships. For example, cucumber mosaic virus strains differ in their ability to multiply in plants at 37° C. Some strains multiply in inoculated leaves and produce systemic symptoms in plants at this temperature; plants systemically infected with one such strain remained infected after prolonged exposure to 37° C. Some other strains appeared incapable of multiplying in inoculated leaves at 37°, and in the case of these strains heat treatment was successful in freeing plants from infection. Tests with one strain of each type showed that both were rapidly inactivated in expressed sap at 37°.

Keeping plants at a high temperature after inoculation

reduces the number of local lesions with some strains of cucumber mosaic virus. Strains, giving rise to necrotic lesions on the inoculated leaves of *Phaseolus vulgaris* var. Canadian Wonder, produced fewer lesions in plants kept after inoculation at 25° C. for 24 hours, and then at 15°, than in plants kept continuously at the lower temperature (Hitchborn, 1956).

Similarly, it has been shown that different strains of another virus, tobacco ringspot, may behave differently in plants at 36° C., some being able and some unable to multiply (Hitchborn, 1957).

The effect of shading and of temperature upon the expression of symptoms in cereals infected with barley yellow-dwarf virus has been studied (Endo, 1957). Shading oats, barley and wheat plants increased the incubation period and decreased the severity of the disease. Shading caused very marked differences in the length of incubation period and in symptom severity in plants infected with a mild strain and intermediate effects in plants infected with a highly virulent strain. Symptoms were severe at 65° and 75° F. and progressively less severe at 82° and 88° F. A highly virulent strain not only caused symptoms sooner than did the mild strain but it killed oat and barley plants at 65°, 75° and 82° F.; only moderately severe symptoms developed at 88° F. With the mild strain good symptoms developed at 65° and 75° F. More severe symptoms were produced with all strains when nights were cool than when nights were warm.

The effects of air temperature and length of day during the incubation period of several viruses affecting leguminous plants have been determined. Plants inoculated with each virus were kept at 15°, 20°, or 25° C. and given 4, 8, or 16 hours of light per day. After the test period all the plants were treated similarly. It was found that the effects of the treatment varied with the virus and the host. For example, alfalfa mosaic virus infected the largest percentage of plants of alsike and crimson clover at 25° C. and 16 hours of sunlight, of sweet clover at 20° C. and 16 hours of

sunlight and of red clover at 15° C. and 16 hours of sunlight. On the other hand, red clover vein-mosaic virus infected the largest percentage of plants of alsike and sweet clover at 20° C. and 16 hours of sunlight, but the largest percentage of red clover plants were infected at 20° and 4 hours of sunlight (Hagedorn & Hanson, 1957).

The light intensity to which plants are exposed *after* inoculation with certain viruses seems to have little effect on the numbers of local lesions produced. The degree of illumination of plants *before* inoculation, however, has considerable effect and the number of lesions is greatly increased by placing the plants before inoculation for 24–72 hours in the dark or by shading for longer periods. This was demonstrated by Bawden & Roberts (1947, 1948) with tobacco mosaic virus (TMV) and tomato bushy-stunt virus (TBSV) on *Nicotiana glutinosa* and with a tobacco necrosis virus (TNV) on tobacco or *Phaseolus vulgaris*.

The effect of this darkening on the susceptibility of plants to infection with viruses was investigated by Wiltshire (1956), who attempted to relate the change in susceptibility to changes in some organic acids. Darkening leaves decreases their content of malic, fumaric, succinic and glycolic acids and increases the content of citric acid. The effect on susceptibility of individual acids infiltrated into the leaf was measured in leaves kept in the light or in the dark before inoculation. None of the acids used produced any large change in susceptibility.

It was found, however, that plants in full light become more susceptible if carbon dioxide is removed from the air, whereas the susceptibility of plants in the dark is not altered. This would suggest that the resistance may be related more closely to photosynthetic carbon fixation, but the evidence is not conclusive.

The nutrition of a plant can affect virus symptomatology, susceptibility to infection and concentration of virus. In the case of potato leaf-roll symptom development is delayed by high temperatures, low soil moisture and high nitrogen. Moreover, the combination of high temperature

and high nitrogen may completely suppress symptom development (Felton 1948).

In some experiments carried out by Wilson (1955) an increased nitrogen supply to potato plants infected with leaf-roll virus greatly reduced the intensity of leaf symptoms, and caused a smaller and less-consistent reduction in phloem necrosis. Application of phosphatic fertilizer initially reduced leaf symptoms, but later increased them; it increased phloem necrosis at all stages of growth. Applications of potash slightly intensified leaf symptoms but had no effect on the phloem necrosis.

The masking of leaf symptoms by nitrogen, and by phosphorus in the early stages of growth, was closely correlated with increases in relative leaf-growth rate produced by these two nutrients.

These experiments also suggested that the two symptoms of leaf-rolling and phloem necrosis were independent of each other since they were differentially affected by nutrient supply and each could occur without the other. This is contrary to the earlier view that the phloem necrosis was the primary symptom of potato leaf-roll and that the rolling of the leaves was consequent to the carbohydrate accumulation caused by the blocked sieve tubes.

The effect on the symptom picture by changing the species of the plant host is quite profound with some species and this emphasizes the futility of any system of classification of viruses based on their symptomatology alone.

This variability in host reaction is well shown by the potato viruses and was the cause of much confusion in the study of this group in the early days. Here we have a great multiplicity of symptoms shown to infection with a single virus, not by different host species, but merely by different varieties of the same species, *Solanum tuberosum*. Thus a virus may give rise to a mottling on one potato variety, a necrotic disease on a second, whilst it may be carried without symptoms by a third. Added to this is the complication of virus strains which differ in virulence and consequently in their symptomatology.

Other viruses may differ in the effect they produce on different plant hosts, thus the virus of tomato spotted wilt (TSWV) gives rise to a characteristic bronze sheen on the leaves of tomato plants. On some other plants, however, notably *S. capsicastrum*, the chief symptom is a bizarre pattern of concentric rings. Again, some of the ringspot viruses, so called, produce their rings on the leaves of some plants but only a mosaic mottle on others.

An extreme example of this type of symptom variation is given by the virus of tomato black ring (TBRV). On tomato, as the name implies, the chief symptom is the development of numerous small black rings on the younger leaves. On tobacco, it is a typical ringspot virus producing numerous clear-cut necrotic rings; on cucumber plants of the outdoor or ridge variety it gives rise to a typical mosaic. But when inoculated to the hot-house variety of cucumber, Telegraph, there is no mottling, but instead large outgrowths, enations, develop on the undersides of the leaves. Furthermore, a number of miscellaneous plants become infected with TBRV but carry it without visible symptoms.

The presence of a concealed or latent virus infection in a host plant may govern the resulting disease. Thus some potato varieties carry the virus X without symptoms, but if they become infected with potato virus Y the resulting disease, called crinkle, is more severe than that produced by either virus acting alone.

The age of a plant at the time of infection sometimes has a bearing on the type of symptom picture which develops. It is of course more difficult to infect an old plant than a young seedling, and not only is the resulting disease less severe but the virus may even enter the plant without producing symptoms at all; this is a frequent occurrence with the tomato black-ring virus.

REFERENCES

BAWDEN, F. C. and ROBERTS, F. M. (1947). 'The influence of light intensity on the susceptibility of plants to certain viruses.' *Ann. appl. Biol.* **34**, 286–96.

(1948). 'Photosynthesis and predisposition of plants to infection with certain viruses.' *Ann. appl. Biol.* **35**, 418–28.

BAWDEN, F. C. and SHEFFIELD, F. M. L. (1939). 'The intracellular inclusions of some plant virus diseases.' *Ann. appl. Biol.* **26**, 102–15.

BEALE, H. A. PURDY (1937). 'Relation of Stanley's crystalline tobacco virus protein to intracellular crystalline deposits.' *Contr. Boyce Thompson Inst. Pl. Res.* **8**, 415–31.

ENDO, R. M. (1957). 'The effect of shading and of temperature upon the expression of symptoms in cereals infected with barley yellow dwarf virus.' *Phytopathology* (abstr.), **47**, 520.

FELTON, M. W. (1948). 'The effect of temperature, moisture and nitrogen on the development of leaf-roll symptoms in the Irish potato.' *Amer. Potato J.* **25**, 50–51.

GRAINGER, J. (1936). 'Low-temperature masking of tobacco mosaic symptoms.' *Nature, Lond.* **137**, 31.

HAGEDORN, D. J. and HANSEN, E. W. (1957). 'Effects of post-inoculation environment on virus infections in clovers.' *Phytopathology* (abstr.), **47**, 522.

HITCHBORN, J. H. (1956). 'The effect of temperature on infections with strains of cucumber mosaic virus.' *Ann. appl. Biol.* **44**, 4, 590–8.

(1957). 'The effect of high temperature on the multiplication of two strains of tobacco ringspot virus.' *Virology*, **3**, 1, 243–4.

IWANOWSKY, D. (1903). 'Über die Mosaikkrankheit der Tabakpflanze.' *Z. Pflanzenkr.* **13**, 1–14.

KASSANIS, B. (1939). 'Intranuclear inclusions in virus-infected plants.' *Ann. appl. Biol.* **26**, 705–9.

KASSANIS, B. and SHEFFIELD, F. M. L. (1941). 'Variations in the cytoplasmic inclusions induced by three strains of tobacco mosaic virus.' *Ann. appl. Biol.* **28**, 360–7.

KUNKEL, L. O. (1921). 'A possible causative agent for the mosaic disease of corn.' *Hawaiian Sug. Plant. Assoc. Exp. Sta. Bot. Ser. Bul.* **3**, 1–14.

KUNKEL, L. C. (1926). 'Studies on aster yellows.' *Amer. J. Bot.* **13,** 646–705.

(1948). 'Studies on a new corn virus disease.' *Archiv. für Virusforsch.* Bd. IV, 24–46.

LITTAU, C. VIRGINIA and BLACK, L. M. (1952). 'Spherical inclusions in plant tumours caused by a virus.' *Amer. J. Bot.* **39,** 87–95.

McWHORTER, F. P. (1941). 'Isometric crystals produced by *Pisum Virus 2* and *Phaseolus Virus 2*.' *Phytopathology*, **31,** 760–1.

MATZ, J. (1919). 'Infection and nature of the yellow stripe disease of cane.' *J. Dept. Agric. Puerto Rico*, **3,** 65–82.

MAYER, G. E. (1882). Over de mosaikziekte van de tabac. *Woorloopie Meded. Landbou. Tidschr.* 359–64.

MILIČIČ, D. and PLAVŠIĆ, B. (1956). 'Eiweisskristalloide in Kakteen-Virusträgern.' *Protoplasma*, **46,** 547–55.

POUND, G. S. (1945). 'Effect of air temperature on the concentration of certain viruses in cabbage.' *J. agric. Res.* **71,** 471–85.

(1952). 'Relations of air temperature and virus concentration to mosaic resistance in cabbage.' *Phytopathology*, **42,** 2, 83–88.

POUND, G. S. and WALKER, J. C. (1945*a*). 'Differentiation of certain crucifer viruses by the use of temperature and host immunity reactions.' *J. agric. Res.* **71,** 6, 255–78.

(1945*b*). 'Effect of air temperature on the concentration of certain viruses in cabbage.' *J. agric. Res.* **71,** 471–85.

ROSENZOPF, E. (1951). 'Sind Eiweissspindeln Virus-Einschlusskörper?' *Ber. ges. Biol. A.* **75,** 3–4.

RUBIO, H. (1950). 'Estudios sobre inclusiones intracelulares producidas por virus en las plantas.' *Microbiol. Espanola*, **3,** 207–32.

(1956). 'Origin and composition of cell inclusions associated with certain tobacco and crucifer viruses.' *Phytopathology*, **46,** 553–6.

RUBIO, H. and VAN SLOGTEREN, D. H. M. (1956). 'Light

and electron microscopy of X-bodies associated with broad-bean mottle virus and *Phaseolus Virus 2*.' *Phytopathology*, **46**, 401–2.

SHEFFIELD, F. M. L. (1931). 'The formation of intracellular inclusions in solanaceous hosts infected with aucuba mosaic of tomato.' *Ann. appl. Biol.* **18**, 471–93.

(1939). 'Micrurgical studies on virus-infected plants.' *Proc. roy. Soc.* Ser. B, **126**, 529–38.

SMITH, K. M. (1924). 'On a curious effect of mosaic disease upon the cells of the potato leaf.' *Ann. Bot.* **38**, 385.

(1958). 'Virus inclusions in plant cells.' *Protoplasmatologia*, **IV**, Virus, 5–16.

STANLEY, W. M. (1935). 'Isolation of a crystalline protein possessing the properties of tobacco mosaic virus.' *Science*, N.S. **81**, 644–5.

STEERE, R. L. and WILLIAMS, R. C. (1953). 'Identification of crystalline inclusion bodies extracted intact from plant cells infected with tobacco mosaic virus.' *Amer. J. Bot.* **40**, 81–84.

THALER, I. (1956). 'Proteinspindeln und anormale Zellwandbildung in der Epidermis viruskranker. *Impatiens holstii-Pflanzen*.' *Protoplasma*, **46**, 755–61.

WALKER, J. C. and LARSON, R. H. (1939). 'Yellow dwarf of potato in Wisconsin.' *J. agric. Res.* **59**, 259–80.

WEBER, F., KENDA, G. and THALER, I. (1952). 'Viruskörper in Kakteen-Zellen.' *Protoplasma*, **41**, 277–86.

WILSON, J. H. (1955). 'Effects of nutrition and light intensity on symptoms of leaf-roll virus infection in the potato plant.' *Ann. appl. Biol.* **43**, 2, 273–87.

WILTSHIRE, G. H. (1956). 'The effect of darkening in the susceptibility of plants to infection with viruses. I. Relation to changes in some organic acids in the French bean. II. Relation to changes in ascorbic acid content of French bean and tobacco.' *Ann. appl. Biol.* **44**, 2, 233–48 and 249–55.

WOODS, M. W. and ECK, R. V. (1948). 'Nuclear inclusions produced by a strain of tobacco mosaic virus.' *Phytopathology*, **38**, 852–6.

CHAPTER III

Methods of Dissemination of Plant Viruses

There are five main methods of natural transmission of plant viruses, but these methods do not of course apply to all viruses, and a very great variation exists among them.

Transmission through the seed plays a comparatively small part in dissemination, but it is important in the spread of a few viruses. Vegetative propagation, on the other hand, is one of the chief methods of perpetuating virus diseases in such crops as potatoes, soft fruits and bulb plants. A certain number are spread by mechanical means, such as contact between the leaves of diseased and healthy plants.

Until recently, transmission through the soil was regarded as a rare phenomenon, but now several new examples have been recorded and it seems likely that quite a number of viruses are soil-borne.

By far the most important method of natural spread is by the agency of insects and other arthropods, and this relationship is discussed at some length in Chapter IV.

Finally, there is the mainly artificial method of grafting by which all viruses which are systemic in their hosts can be disseminated. The words 'mainly artificial' are used advisedly because natural grafts between woody plants do occur, and because many viruses can be transmitted by means of the parasitic plant, *Cuscuta* spp., which by linking together plants of very different species forms a kind of natural graft.

SEED-TRANSMISSION

There is scope for considerable investigation into the

phenomenon of seed-transmission of viruses since knowledge of this subject is very meagre. It is not known why so few viruses are seed-borne though the anatomical isolation of the embryo is usually quoted as the reason.

In lettuce mosaic the proportion of infected seed may vary considerably; in one variety of lettuce the percentage of seeds infected with mosaic virus varied from 0·2 to 14·2. Plants infected just before flowering produce fewer infected seeds than those infected when young and plants infected after flowering produce none. In some lettuce varieties the virus is not seed-transmitted at all, and this is governed by the particular response of the plant to infection. An example of this is given by the variety Cheshunt Early Giant because the first formed floral heads are killed by infection and any secondary shoots formed contain little virus. The lettuce mosaic virus is also passed through the seed of groundsel, *Senecio vulgaris* L., which rarely shows symptoms but from which the virus may be transmitted by aphids to lettuce (Broadbent, 1958).

Some interesting studies on the seed-transmission of the virus causing false stripe disease of barley have been carried out. Rod-shaped particles measuring 30 mμ × 130 mμ have been found by electron microscopy to be associated with the disease. These particles occur in large numbers, and are found in the leaves, embryos, endosperm, pollen and unfertilized pistils. Pollen-transmission of the disease was suggested by the presence of the rods in seed produced from healthy pistils pollinated by pollen from diseased plants. Seed from diseased pollen and healthy pistils produced a small percentage of diseased seedlings (Gold, Suneson, Houston & Oswald, 1954).

Transmission of a virus through the seed of one host species but not through the seed of another also occurs. There are several examples of this; the virus of cucumber mosaic is transmitted through the seed of the wild cucumber (*Micrampelis lobata*) but not apparently through the seed of the cultivated cucumber (Doolittle & Walker, 1925). Mahoney (1935) offers evidence that the virus is also

carried in the seed of certain inbred lines of muskmelon, *Cucumis melo* L.

The virus of cowpea mosaic is transmitted by the seed of the asparagus bean, a variety of *Vigna unguiculata*, but not by the seed of the cowpea. Similarly the tobacco ringspot virus is not carried in the seed of the tobacco plant but is present in the seed of *Petunia* sp. (Henderson, 1931) and of the Lincoln variety of soybean (Desjardins, Latterell & Mitchell, 1954).

Other examples are the transmission of *Abutilon* mosaic virus through the seed of certain species only (Keur, 1933), of the dodder latent mosaic virus through the seed of *Cuscuta campestris* but not through the seed of cantaloupe, buckwheat and pokeweed, *Phytolacca americana* L. (Bennett, 1944), and of tomato ringspot virus through the seed of the Lincoln variety of soybean (Kahn, 1956).

VEGETATIVE PROPAGATION

If a plant is virus-infected all the vegetative parts used to propagate it will contain the virus so that new plants produced by this method will also be virus-infected. It is for this reason that virus diseases are of such paramount importance in the potato crop, in raspberry and strawberry culture, in the bulb industry and many other crops which are produced from vegetative parts.

There may be a few exceptions to this rule; where the virus is not completely systemic in the host it is sometimes possible to obtain virus-free cuttings. If potato tubers are harvested whilst still immature from a plant recently infected, the virus may not have reached all the tubers. In potato plants infected with the virus of tomato spotted wilt, a percentage of the tubers seem to be virus-free.

DISSEMINATION BY MECHANICAL MEANS

There are various types of dissemination by mechanical means chiefly applicable to viruses which are in high con-

centration in the plant; first, some viruses can spread from a diseased plant to a healthy one by contact of the leaves brought about by the wind. This is thought to be the main method of spread of potato virus X, the most ubiquitous and widely disseminated of all plant viruses. The virus of potato spindle tuber is stated to be carried from plant to plant in the same way.

Broadbent (1957) has shown by means of experiments with a wind tunnel that the viruses of turnip yellow mosaic and turnip crinkle will also spread in this manner.

Cultivation procedures and the movement of animals may play some part in the spread of viruses. According to Todd (1958), potato virus X can persist for as long as 6 weeks on the clothing of potato inspectors and of those who cultivate or spray the crop. Dogs and rabbits may also carry the virus on their feet and limbs. See also Bercks (1956). Tractors are said to infect 4–12 per cent. of healthy potato plants with the virus of spindle tuber during cultivation and the knife used for cutting the seed pieces will also spread this virus (Merriam & Bond, 1954). Potato virus X is not spread by the seed-cutting knife unless possibly when it passes through an eye. Tulip break virus is sometimes carried on the knife used to cut blooms, but this does not occur with the virus of narcissus stripe (van Slogteren & Ouboter, 1941). Similarly the virus of cymbidium mosaic is readily spread by pruning shears (Jensen & Gold, 1955). Potato virus X may also be spread among potatoes in sacks by contact of the sprouts (Bawden, Kassanis & Roberts, 1948).

Some viruses may spread below ground by mechanical contact between the roots of infected and healthy plants. This was demonstrated by Roberts (1948, 1950) with potato X virus in potatoes and tomatoes, the spread being much more rapid by the roots of the tomato plants. Klinkowski (1951) has also obtained positive results in similar experiments with potato virus X. On the other hand, the virus of narcissus stripe cannot be disseminated in this way even when the roots are intertwined.

SOIL TRANSMISSION

At one time the view was current that very few plant viruses were transmitted through the soil, and this view, firmly held as it was, tended to obstruct progress in this particular field. Recently work by Cadman (1956) and Harrison (1956, 1957) has suggested the existence of quite a number of soil-borne viruses.

Tobacco mosaic virus was the first case of soil transmission known since it was pointed out by Mayer in 1886 that the disease could be contracted from the soil. However, this is really a case of mechanical transmission since the virus is known to retain its infectivity in fragments of infected plants for long periods. There are, however, other cases of relatively unstable viruses where there is probably a more intimate relationship between the soil and the virus, though we know very little at present as to the nature of this relationship.

An early discovered example of a truly soil-borne virus is that of wheat mosaic (Webb, 1927, 1928), and another example is that of the virus causing the mosaic disease of oats (McKinney, 1946). An attempt has been made recently to discover something of the mechanism involved in the spread of these two viruses. Certain chemicals such as formaldehyde, chloropicrin, carbon bi-sulphide and ethyl alcohol easily rendered infected soils non-infectious; but toluene had no effect. When plants were grown in autoclaved soil, to which roots from naturally infected plants from the field had been added, they became infected. This did not occur, however, when the soil was inoculated with virus-infected sap or leaves, or with roots or leaves from plants infected mechanically. The suggestion is therefore made that a vector, closely associated with the roots of mosaic-diseased plants, is involved in the overseasoning and transmission of the soil-borne viruses affecting cereals (McKinney, Paden & Köhler, 1957).

Some further attempts to discover what the vector, if such exists, might be have been made by McKinney *et al.*

(1957). Virus-free cereal plants were grown in a sand culture and then mechanically inoculated; when the plants were infected the aerial parts were removed and fresh cereal seed already germinated was planted in the remaining root culture. If the organism to be tested is a vector, then the subsequent plants arising from the seed will develop mosaic. So far, negative results have been obtained in experiments with two species of nematode.

Another soil-borne virus which has a very wide distribution causes the tobacco 'rattle' disease, stem-mottle of potato and 'malaria' of hyacinths. Here again soil-inhabiting organisms are being tested as vectors. Another possibility is that the viruses may be adsorbed by clay minerals and so retain infectivity, but it is a striking fact that many of the soil-borne viruses are not particularly stable and would be unlikely to retain infectivity for long if merely left free in the soil (van der Want, 1952).

An interesting point in connexion with soil-borne viruses is the apparent relationship with the soil texture, some apparently being associated with light, and others with heavy, soils.

GRAFTING

All viruses which are systemic in their hosts can be transmitted by grafting between susceptible and compatible plants. There are many methods of making grafts but only a few, which are suitable for use with herbaceous plants are given here.

In the method of *detached scion grafting* the wedge or cleft graft is commonly used; a small shoot is trimmed to a wedge and inserted in the cut stem of the stock; this cut should be made through a node if possible, otherwise the stem may be hollow. The scion should have most of its leaves removed and it should be inserted in the cleft of the stock so that the apical portions of the cut surfaces of the scion are just visible. The graft is then bound with bast, or preferably thin rubber tape, and sealed with a drop of

bicycle tyre solution. The plant should then be placed in a moist atmosphere for a few days; it is not necessary to remove the rubber tape since by the time a graft union is formed the rubber tape will have perished and fallen away. This method of grafting is very suitable for studying the virus diseases of the potato, the tomato and tobacco plants, and it is usual for the scion to be virus-infected rather than the stock.

The other method of grafting suitable for herbaceous plants is known as *approach grafting*. In this type the plants to be joined are brought together, but each retains parts above and below the point of contact. The simplest application of this method is known as the spliced approach graft. The stock and scion are each sliced to expose the cambium; the cut surfaces are then brought together and tied.

The tongued approach graft is a suitable one for studying virus transmission and has been used by Harris (1932) and by Harris & King (1942) for investigating the virus diseases of strawberries. A 'tongue' is cut downwards on the stock and upwards on the scion; the tongues are then fitted together and wrapped with self-sealing crepe rubber.

Another method is known as cleft inarching; an upward cut in the stock forms the cleft and the scion is cut to a wedge to fit into the cleft. The whole must them be firmly tied.

A useful method is the bottle graft; here the leafy scion is approach-grafted to the stock, but the base of the scion is kept alive by immersion in a bottle of water until union is established, when the base of the scion is cut off close to the stock.

Modifications in grafting methods for studying the virus diseases of strawberries have been made by Bringhurst & Voth (1956). They cleft-grafted excised terminal leaflets from test plants to the petioles of indicator plants (*Fragaria vesca*). The scion leaflet is reduced by two-thirds, and the petiole, trimmed to a wedge, is inserted in a split in the stock petiole between the laterals, the terminal leaflet having been removed. Successful graft unions are quickly

detected and the symptoms of virus disease develop within two to five weeks in *F. vesca*. Leaves can be grafted immediately after collecting in the field or stored in polythene bags for a month or more at 36° F. before grafting.

Miller (1958) has studied the comparative efficiency of excised leaf-petiole grafts and stolon grafts for transmitting certain strawberry viruses. It was found that a prepared scion inserted in a slit made in the stock petiole, just below the lateral leaflets, without removing the terminal one, was as efficient as stolon grafting (approach method) in transmitting the strawberry mottle, veinbanding, latent and mild crinkle virus components to *Fragaria vesca* × *F. Vesca* var. *Alpina* indicator plants. Symptoms usually appeared 5–10 days earlier and were more severe.

Tuber grafting is sometimes useful for the transmission of some potato viruses. Cork borers of different sizes are used for this purpose; a core containing an eye is removed from the infected tuber and placed in the socket made in a healthy tuber by removing a plug with a cork borer one size smaller.

Tulip viruses can sometimes be transmitted by binding together infected and healthy halves of tulip bulbs.

For a comprehensive account of most known methods of grafting the reader is referred to Garner (1958).

A form of grafting by using the parasitic plant dodder, *Cuscuta* spp., is sometimes useful, especially in transferring viruses to plants in which they can be more easily studied. Schmelzer (1956) has recently carried out some experiments to determine the suitability of nine species of dodder as virus carriers. *Cuscuta campestris*, *C. californica*, *C. subinclusa*, *C. europaea* and *C. epithymum* were almost uniformly effective in the transmission of the ordinary green strain of cucumber mosaic virus to *Nicotiana glutinosa*, but only 6 out of 40 tests with *C. lupuliformis* gave positive results.

Lucerne (alfalfa) mosaic virus was transmitted to tobacco, var. *Samsun*, by *C. campestris*, *C. subinclusa* (red and white leaved), *C. europaea* and *C. epilinum* did not act as carriers

either in this test or in test with potato stem mottle. *C. campestris*, *C. gronovii* and *C. lupuliformis* were the most effective carriers of potato stem mottle virus to tobacco, whilst tobacco etch virus was transmitted to tobacco only by *C. lupuliformis*. All the species of dodder gave negative results with bean yellow mosaic virus, potato virus Y and potato bouquet virus. Tomato spotted wilt virus was transmitted fairly well by *C. californica* to *N. glutinosa* and tobacco mosaic virus to tobacco by *C. campestris*.

The virus of chrysanthemum flower distortion can be transmitted by *C. campestris* to periwinkle, *Vinca rosea* (Brierley & Smith, 1957).

REFERENCES

BAWDEN, F. C., KASSANIS, B. and ROBERTS, F. M. (1948). 'Studies on the importance and control of potato virus X.' *Ann. appl. Biol.* **35**, 2, 250–65.

BENNETT, C. W. (1944). 'Latent virus of dodder and its effect on sugar beet and other plants.' *Phytopathology*, **34**, 1, 77–91.

BERCKS, R. (1956). 'On the concentration and behaviour of the X-virus in old leaves.' *Phytopath. Z.* **26**, 1, 35–40.

BRIERLEY, P. and SMITH, F. F. (1957). 'Symptoms of chrysanthemum flower distortion, dodder transmission of the virus, and heat cure of infected plants.' *Phytopathology*, **47**, 448–50.

BRINGHURST, R. S. and VOTH, M. (1956). 'Strawberry virus transmission by grafting excised leaves.' *Plant Dis. Reptr.* **40**, 596–600.

BROADBENT, L. (1957). *Investigations of Virus Diseases of Brassica Crops*. London and New York: Cambridge Univ. Press.

(1958). 'The spread and control of plant viruses.' *Sci. Hort.* **13**, 74–79.

CADMAN, C. H. (1956). 'Studies on the etiology and mode of spread of raspberry leaf curl disease.' *J. hort. Sci.* **31**, 2, 111–18.

DESJARDINS, P. R., LATTERELL, R. L., MITCHELL, J. E. (1954). 'Seed transmission of tobacco ringspot virus in Lincoln variety soybean.' *Phytopathology*, **44**, 2, 86.

DOOLITTLE, S. P. and WALKER, M. N. (1925). 'Further studies on the overwintering and dissemination of cucurbit mosaic.' *J. agric. Res.* **31**, 1–58.

GARNER, R. J. (1958). *The Grafter's Handbook*, pp. 1–260. London: Faber & Faber.

GOLD, A. H., SUNESON, C. A., HOUSTON, B. R., and OSWALD, J. W. (1954). 'Electron microscopy and seed and pollen transmission of rod-shaped particles associated with the false stripe virus disease of barley.' *Phytopathology*, **44**, 115.

HARRIS, R. V. (1932). 'Grafting as a method for investigating a possible virus disease of the strawberry.' *J. Pomol.* **10**, 35–41.

HARRIS, R. V. and KING, M. E. (1942). 'Studies in strawberry virus diseases. V. The use of *Fragaria vesca* L. as an indicator of yellow-edge and crinkle.' *J. Pomol.* **19**, 227–42.

HARRISON, B. D. (1956). 'Soil-transmission of Scottish raspberry leaf-curl disease.' *Nature, Lond.* **178**, 553.

(1957). 'Studies on the host range, properties and mode of transmission of beet ringspot virus.' *Ann. appl. Biol.* **45**, 462–72.

HENDERSON, R. G. (1931). 'Transmission of tobacco ringspot by seed of petunia.' *Phytopathology*, **21**, 2, 225–9.

JENSEN, D. D. and GOLD, A. H. (1955). 'Hosts, transmission, and electron microscopy of Cymbidium mosaic with special reference to Cattleya leaf necrosis.' *Phytopathology*, **45**, 6, 327–34.

KAHN, R. P. (1956). 'Seed transmission of the tomato ringspot virus in the Lincoln variety of soybeans.' *Phytopathology*, **46**, 5, 295.

KEUR, J. Y. (1933). 'Seed transmission of the virus causing variegation of *Abutilon*. *Phytopathology* (abstr.), **23**, 1, 20.

KLINOWSKI, M. (1951). 'A contribution to the question of the possibility of infection by potato virus X through root contact.' *Z. PflKrankh.* **58,** 1–2, 3–6.

MCKINNEY, H. H. (1946). 'Mosaics of winter oats induced by soil-borne viruses.' *Phytopathology*, **36,** 5, 359–69.

MCKINNEY, H. H., PADEN, W. R. and KOEHLER, B. (1957). 'Studies on chemical control and overseasoning of, and natural inoculation with, the soil-borne viruses of wheat and oats.' *Plant Dis. Reptr.* **41,** 256–66.

MAHONEY, C. H. (1935). 'Seed transmission of mosaic in inbred lines of muskmelons (*Cucumis melo* L.).' *Proc. Amer. Soc. Hort. Sci.* **32,** 477–80.

MERRIAM, D. and BONDE, R. (1954). 'Dissemination of spindle tuber by contaminated tractor wheels and by foliage contact with diseased potato plants.' *Phytopathology* (abstr.), **44,** 2, 111.

MILLER, P.W. (1958). 'Comparative efficiency of excised leaf petiole grafts and stolon grafts for transmitting certain strawberry viruses.' *Plant Dis. Reptr.* **42,** 1043–7.

ROBERTS, F. M. (1948). 'Experiments on the spread of potato virus X between plants in contact.' *Ann. appl. Biol.* **25,** 2, 266–78.

(1950). 'The infection of plants by viruses through roots.' *Ann. appl. Biol.* **37,** 3, 385–96.

SCHMELZER, K. (1956). 'Beiträge zur Kenntnis der Übertragbarkeit von Viren durch cuscuta-Arten.' *Phytopath. Z.* **28,** 1–56.

TODD, J. M. (1958). 'Spread of potato virus X over a distance.' *Proc. 3rd Conf. Pot. Virus Dis.*, Wageningen-Lisse, 1957, 132–43.

VAN DER WANT, J. P. H. (1952). 'Some remarks on a soil-borne potato virus.' *Proc. Conf. Pot. Virus Dis.*, Wageningen-Lisse, 1951, 71–75.

VAN SLOGTEREN, E. and OUBOTER, M. P. DE B. (1941). 'Onderzoekingen over virus-ziekten in bloembolgewassen I. Narcissen. I.' *Meded. Landbouwhoogeschool* Wageningen, **45,** 32 pp.

WEBB, R. W. (1927). 'Soil factors influencing the development of the mosaic disease of winter wheat.' *J. agric. Res.* **35**, 587–614.

(1928). 'Further studies on the soil relationship of the mosaic disease of winter wheat.' *J. agric. Res.* **36**, 53–76.

CHAPTER IV

Relationships of Plant Viruses with their Arthropod and Other Vectors

INTRODUCTION

The relationship between viruses, and especially plant viruses, and the insects which transmit them (vectors) is a complicated and interesting one. Although a great many facts have been collected we are still very far from a clear understanding of the situation.

A suspicion that there was some sort of connexion between certain diseases of plants and insects is more or less coincident with the discovery of the first virus in 1892. Towards the end of the nineteenth century, Takata (1895), in Japan, showed that the dwarf disease of rice was due to something put into the plant by the leafhopper, *Deltocephalus dorsalis* Motsch. However, this work did not become available to Western workers for many years and, in the meantime, Ball in 1909 had connected the leafhopper *Circulifer* (*Eutettix*) *tenellus* with the disease of sugar beet called curly-leaf, better known as curly-top. A year later Shaw (1910) demonstrated that this leafhopper did in fact infect healthy sugar beet with the curly-top disease. Allard in 1914 is generally credited with the discovery that aphids could transmit plant viruses, and he claimed to have shown that an aphid, known as *Macrosiphum tabaci*, was the vector of tobacco mosaic virus. We know now, however, that aphids do not transmit this virus, which does not, apparently, have an insect vector. Possibly Allard was working with a strain of cucumber mosaic virus which can simulate very closely the symptoms of tobacco mosaic

virus in tobacco. In 1916 Doolittle and Jagger, working independently, showed that *Aphis gossypii* could transmit the virus of cucumber mosaic.

In 1918 Doolittle & Gilbert transmitted what they thought was cucumber mosaic virus by means of the striped cucumber beetle. There is, however, no case known of an aphid-transmitted virus being carried by a beetle or other mandibulate insect, and vice versa. The probable explanation is that Doolittle & Gilbert were working with one of the group of squash-mosaic viruses which are beetle-transmitted as opposed to the aphid-transmitted viruses of the cucumber mosaic group.

Now there are over 300 separate plant viruses described, not all of which by any means are known to have insect vectors. More viruses are transmitted by aphids than by any other type of insect, and one aphid species in particular, *Myzus persicae* Sulz., is known to transmit nearly fifty separate viruses. Next in importance come the leafhoppers, which are the vectors of many important virus diseases in the Americas. Until just recently no leafhopper-borne viruses were known in western Europe, but now at least three have been described and more no doubt will be discovered in the near future. A number of species of scale insects, Coccoidea, are concerned in the spread of one particular group of viruses affecting cocoa trees (*Cacao*). There is one virus carried by thrips, that of tomato spotted wilt, and there are nine viruses transmitted by whiteflies (Aleurodidae). Three or four viruses have beetles as vectors and four viruses are borne by Eriophyid mites.

The various kinds of relationships between the viruses and these different types of vectors are discussed in the remainder of this chapter.

DIFFERENT TYPES OF INSECT-VIRUS RELATIONSHIPS

Mechanical Transmission

In this context mechanical transmission involves the purely passive transfer to the plant of virus contaminating the

mouthparts of the insect in question. Unexpectedly enough, there appear to be very few instances of this method of virus spread. We have mentioned earlier that no insect vector is known for the virus of tobacco mosaic, a curious fact in view of the extremely infectious nature of this virus. However, this statement needs qualifying slightly because Walters (1952) has shown that the virus can be transmitted by an insect under rather artificial conditions. In his experiments Walters used the large grass-hopper, *Melanoplus differentialis;* he allowed the insects to feed on a mosaic tobacco plant and then transferred them immediately to healthy tobacco plants. He did the same thing with two other sap-inoculable viruses, that of tobacco ringspot and potato virus X. In each case some successful infections resulted by mechanical transfer of virus, residual on the mouthparts, to the healthy plants during the process of eating the leaves.

Some controversy has centred round the question whether aphids are ever mechanical vectors of plant viruses, as first suggested by Doolittle & Walker (1928). There are several factors which seem to militate against this theory. For example, the specificity of transmission shown by aphids, in which one species can transmit a virus and another species is unable to do so, cannot be explained on the basis of a mere mechanical contamination of the mouthparts. Bradley (1952) tried the experiment of contaminating externally the stylets of the aphid *Myzus persicae* with the viruses of henbane mosaic and tobacco mosaic, but failed to obtain positive transmission. Furthermore, Watson & Roberts (1940) point out that the aphid *M. persicae*, if transferred rapidly, can transmit the viruses of henbane mosaic, severe etch and potato virus Y from tobacco to a number of successive healthy plants. This could hardly happen if the transmission depended on the mechanical cleaning of the stylets. On the other hand, this might be possible if, as suggested by Hoggan (1933), small quantities of virus lodged in the ducts *inside* the stylets of the aphids; under these circumstances more than one

feeding puncture might be necessary to remove all the virus. Bradley (1952) suggests that the salivary sheath formed in the plant by the gelling of the salivary material may act as a filter for the aphid's food and its absence during brief feeding punctures may lead to one of the ducts in the stylets becoming obstructed and so cause the aphid to clear it by forcing liquid outwards. This may happen several times, and during the process virus from the previous feeding puncture may pass into the cell.

None of these assumptions, however, explains the non-transmission of certain viruses by aphids nor the specificity of vectors.

Day & Irzykiewicz (1954) have suggested a modified hypothesis of mechanical transmission based on the following points: (1) Short transmission cycle. This may be as short as one minute for both the acquisition and inoculation of virus. (2) Absence of a latent period. (3) Short duration of retention of virus and the rapid loss of infectivity in successive inoculation feeds. (4) Usual absence of vector specificity. (5) Ease with which these viruses can be transmitted mechanically. (6) The absence of retention of virus following a moult. They consider that taken together these six points are conclusive of mechanical transmission.

However, specificity of vectors and selectivity in transmission must still be explained, and Day & Irzykiewicz attempt to do this on the assumption of virus inhibitors in the saliva and the different response of viruses to these inhibitors.

In the writer's opinion, however, the case for the mechanical transmission of plant viruses by aphids has not yet been satisfactorily made out.

Aphid-transmitted Non-persistent Viruses

Aphid-transmitted viruses were first divided into two categories by Watson (1936, 1938) and Watson & Roberts (1939, 1940). 'Non-persistent' viruses are rapidly lost by the vector, usually after a short period of feeding, whilst

'persistent' viruses are retained by the vector for long periods, frequently for the rest of the insect's life, without the necessity for recourse to a fresh source of virus.

A great deal of investigation has been carried out on the conditions governing the non-persistent type of transmission, but the situation is still confused and the exact significance of the various facts is still not understood.

We are assuming, of course, that in a non-persistent virus there is a closer relationship between the virus and the aphid vector than there is in the case of the virus transmitted by purely mechanical means.

The main facts concerning non-persistent viruses in relation to their aphid vectors are as follows:

(1) Vectors are optimally infective when they have fed only a few minutes on the infected plant.

(2) Virus transmission is improved if aphid vectors are starved for a period before an infection (= acquisition) feed.

(3) After acquisition-feeding infectivity is rapidly lost when the vectors feed on healthy plants.

(4) Infectivity is lost much more slowly when the vectors fast after acquisition-feeding.

Watson & Roberts (1939) consider that the most probable explanation of these effects is that the viruses are inactivated by some substance produced by the aphids when feeding. This hypothesis of an inactivating substance produced by aphids only while feeding can be extended to cover most of the experimental facts, including possibly the specificity of transmission. If such a substance is produced, however, there is no information on where it is produced or where it comes into contact with the virus (Bradley, 1952).

The answer may be in a combination of mechanical and inactivator hypotheses. For purposes of discussion Sylvester (1954) makes the following assumptions: (1) Transmission is mechanical in the sense that virus is carried within the food canal of aphids. (2) Aphids feed in a similar manner and in similar areas during initial stages of penetration,

although they may exhibit variations in the rate of penetration. (3) Aphids acquire a similar charge of virus when feeding a short time on a given virus source plant. (4) The action of inactivators which are present in the salivary secretions is not upon the virus but rather upon the host plant cells into which the virus is injected, i.e. the insect renders the host plant cell resistant or practically immune to infection. Sylvester admits that the evidence in support of this 'incompatibility hypothesis' is meagre and goes on to suggest that the implications would be such as to indicate that all viruses such as that of tobacco mosaic, potato virus X, &c., are taken up by the aphids when feeding, but cannot be demonstrated as being in the insects by transmission tests because the combination of salivary secretions and the contents of inoculated cell is incompatible with the virus to such an extent that transmission is highly improbable.

Regarding the question as to whether the aphid actually picks up a virus which it cannot transmit, the evidence is rather contradictory. It was shown by Bennett & Wallace (1938) that the virus of beet curly-top will persist for a number of days in the aphid *Myzus persicae*, although the insect is unable to transmit the virus. On the other hand, a recent paper by van Soest & de Meester-Manger Cats (1956) suggests that the aphid *M. persicae* is unable to imbibe the virus of tobacco mosaic from infected plants of *Nicotiana glauca*. It is known that if the stylets of an aphid are severed during the act of feeding, droplets of sap continue to emerge from the cut ends of the stylets, apparently forced out by the turgidity of the plant. Such droplets were tested for the presence of tobacco mosaic virus by (1) inoculation to test plants, (2) examination under the electron microscope, (3) tests by a method of microserology. All these tests gave negative results and lead the authors to the conclusion that the aphid does not imbibe the virus. They suggest that this seems to support the suggestion made by Sukhov (1944) that the salivary sheath, which surrounds the stylets in the plant, acts as a barrier against

the tobacco mosaic virus. However, the fact that *M. persicae* can easily transmit other rod-shaped viruses such as that of cabbage black ringspot is against Sukhov's hypothesis.

There is slight evidence for the suggestion that inactivators present in the salivary secretions of the aphid act upon the host cell rather than the virus in the work of Kirkpatrick & Ross (1952) on potato leaf-roll. They found that the presence of large numbers of aphids, either infective or non-infective, on a test plant decreased the probability of obtaining an infection, which seems to suggest that the test plant was being modified.

Aphid-transmitted Persistent Viruses

The chief differences between this type of virus and the foregoing non-persistent viruses are, first, the long period of time, frequently the rest of the life of the aphid, during which the insect retains infectivity. Secondly, the delay in the development of infective power within the aphid, which can be put in another way. Thus in progressive transfers of infective aphids from plant to plant, the non-persistent virus is carried to the first plant and rarely to the second if the feeding periods are of some hours' duration, whilst the persistent virus is not transmitted to the first two or three plants but to all the others for a considerable period. Thirdly, there is a greater specificity in the aphid vectors of persistent viruses which are not as a rule transmitted by six or more species as may be the case with some non-persistent viruses. In addition, the majority of persistent viruses are not transmissible by mechanical means and the ability of aphid vectors to transmit this type of virus is not affected by preliminary starving.

The delay in the development of infective power in the aphid or, put more shortly, the latent period, varies greatly with the different viruses. The longest latent period, so far discovered, is that of a strawberry virus known as 'Virus 3' transmitted by the aphid *Capitophorus fragariae* Theob., which takes 10 to 19 days. Aphids left for 16 days on an

infected plant caused infection in the first day of test feeding. It is not known how far the latent period of a persistent virus in the aphid vector is of biological significance, but in some cases it appears to be a function of the test plant. Thus it appears possible to reduce the latent period of the leaf-roll virus in the aphid by the use of host plants of greater suceptibility and in which the virus may be present in higher concentration than in the potato (Kassanis, 1952; Kirkpatrick & Ross, 1952; Klostermeyer, 1953).

The suggestion has been made by Day (1955) that the potato leaf-roll virus may multiply to a limited extent within the aphid. However, experiments carried out by Cadman & Harrison (1956) do not seem to support this. It is known that potato tubers can be freed from leaf-roll virus by keeping them at 36° C. for 3 weeks, and experiments were made to investigate the effects of high temperatures on the persistence of the virus in the aphid *Myzus persicae*. The ability of infective aphids to transmit the virus was greatly decreased by exposing them to 32° C. for 3 or 6 days. The ability to transmit more regularly did not return when the aphids were kept for a further period at 20° C. These results seem to provide no evidence that the virus multiplies in the aphid since, if it did, one would expect the insects to return to their full transmitting power at normal temperatures.

In the complex disease of tobacco known as 'rosette' (Smith & Lea, 1946) the two component viruses are called the 'vein-distorting' and 'mottle' viruses respectively. Experiments show that both viruses persist in the aphid vector for at least 3 weeks, since infection is carried right through twenty serial transfers of 24 hours feeding on each plant. During these transfers occasional plants developed either the mottle disease only or else the vein-distorting disease only, showing that the flow of virus from the insect is not uniform.

Sylvester (1949*a*, *b*) suggests three factors which would aid in classifying a virus as persistent or non-persistent.

These are: (1) The period of retention of the virus by the aphid. (2) The effect of a prefasting period, prior to the infection-feeding, upon subsequent transmission efficiency. (3) The status of sap-transmissibility of the virus. It follows that a typical aphid-borne persistent virus would have these characteristics:

(1) It would be retained by the aphid vector for a relatively long period of time.

(2) The vector efficiency would not be influenced by a prefasting period prior to infection-feeding.

(3) It would not be sap-transmissible with ordinary facility.

A typical non-persistent virus would be opposite in these respects, whilst a virus would be classified as persistent if it had any combination of two or more of the three characteristics in common.

Transmission with Multiplication of Virus in the Insect Vector

The question as to whether a plant virus can multiply in an animal, i.e. its insect vector, has long been debated, since it is obviously of considerable interest and it is only comparatively recently that this question has been decided in the affirmative. It has taken many years and the slow accumulation of evidence to show that certain plant viruses do multiply in a certain type of insect vector.

The first to offer some evidence on this point was a Japanese worker (Fukushi, 1933), who studied the dwarf disease of rice and its leafhopper vector, *Nephotettix apicalis* Motsch. He showed that the virus was transmitted from an infective parent insect to the offspring, but only through the female parent. Moreover, the progeny from such an infected parent did not itself become infective until after a period of 9 days from the date of hatching. Fukushi also showed that the virus could be passed through six generations involving eighty-two infective leafhoppers and all derived from a single virus-bearing female without access to a further source of virus. This is strong indirect evidence of multiplication since otherwise the dilution involved would be too great.

Black (1950) carried out similar experiments to those of Fukushi with the virus of clover club-leaf which he has shown to be transmitted through the egg of the vector, a leafhopper, *Agalliopsis novella* Say. From a pair of viruliferous (= virus-bearing) leafhoppers the breeding was carried out through twenty-one generations over a period of 5 years. The insects were fed throughout on virus-immune lucerne plants without loss of infectivity. Black has calculated that, if multiplication of the virus is not assumed, the dilution of the original virus in the parent insect exceeded $1 : 2{\cdot}8 \times 10^{26}$.

Kunkel (1937) has made an interesting study of the virus of aster yellows and its leafhopper vector, *Macrosteles fascifrons* Stal. He exposed viruliferous leafhoppers to a temperature of 32° C. for varying periods and found that this exposure to high temperatures deprived the insects of their infectivity for a period. The length of this period depended on the length of the exposure to 32° C. If the insects were kept for 1 day at this temperature they regained infectivity within a few hours. If they were kept several days it required 2 days for them to regain infectivity, and if they were kept at 32° C. for 12 days infectivity was entirely lost. Kunkel interpreted these results as indicating that exposure to high temperatures reduced the amount of virus in the insect below the infectivity limit, and the delay before the insect again became infective was necessitated by the multiplication of the virus up to a sufficient concentration for infection.

Long exposure destroyed the virus altogether and so rendered the insect non-viruliferous. The ability of these insects to regain virus from a fresh source of infection was not apparently affected by the heat treatment. It is instructive to compare the results of this experiment with those of a similar experiment, previously described with the aphid *Myzus persicae* and the virus of potato leaf-roll. Here, unlike the leafhopper, the aphid did not regain normal infectivity when returned to the lower temperature

Black (1941) approached the problem from a slightly.

different angle, since he had shown in 1940 that the virus of aster yellows could be inoculated successfully into the leafhopper vector, thus rendering it viruliferous. He colonized a large number of the leafhoppers on a yellowed aster plant for a given time and then removed all the insects to plants of rye which are immune to the aster yellows virus. Thus the leafhoppers received approximately the same dose of virus. Next, a number of the insects were ground up into a paste, made into various dilutions and inoculated into the alimentary canals of virus-free aster leafhoppers. This rather roundabout method had to be employed because the aster yellows virus is not mechanically transmissible to its host plant. Black found that those leafhoppers which had been longest on the rye plants contained most virus since they would withstand the highest dilutions and still produce infectivity in the inoculated leafhoppers, the inference being that the virus had multiplied most in those insects which had remained alive longest after the intake of virus in the first place.

A more direct method of measuring multiplication of a virus in an insect vector is by serial inoculations from insect to insect. This was first done by Merrill & TenBroeck (1934), who demonstrated the multiplication in the mosquito of the virus of equine encephalomyelitis. Maramorosch (1952) applied this technique, and by means of a microsyringe succeeded in carrying the virus serially through ten groups of leafhoppers. He calculated that if the virus was not multiplying the dilution at the tenth passage would have reached 10^{-40}.

In 1938 Trager succeeded in cultivating the virus of equine encephalomyelitis in hanging-drop culture of mosquito tissues, and this technique was applied by Maramorosch (1956) to the virus of aster yellows and its leafhopper vector *Macrosteles fascifrons* Stal. In these experiments nymphs of aster leafhoppers were allowed to feed for 2 days on diseased plants of China aster to acquire aster yellows virus. No virus was recovered when juices of these leafhoppers were injected into virus-free leafhoppers on

the following day. However, virus was recovered by the same injection method after the nymphs has been cut in pieces and their living tissues incubated for 10 days in a suitable medium in hanging drops. This experiment demonstrates that aster yellows virus can complete its incubation not only in vectors feeding on a constant supply of fresh plant food but also in insect tissues *in vitro*. The experiment also provides additional evidence for multiplication of this plant virus in the tissues of its insect vector.

In the case of another leafhopper-borne virus, that of corn (maize) stunt and its vector, *Dalbulus maidis* Del. & Wol., it was observed by Kunkel (1948) that the lengths of the incubation period of the virus in both host plant and insect vector were the same at optimal temperature.

Maramorosch (1951) inoculated the corn stunt virus, at dilutions of 1 : 100 insect pulp, into the leafhoppers and found that approximately 6 weeks elapsed after inoculation before the insects became infective. This indicates that the virus was multiplying in the leafhoppers during this period.

Some doubt exists whether the well-known virus of beet curly-top does multiply in its vector, the leafhopper *Circulifer tenellus*. There is a certain amount of evidence which supports both views. Thus, leafhoppers frequently lose their ability to transmit the virus but can reacquire it from an infected beet. Moreover, the longer the insects fed on a source of virus the longer they remained infective (Bennett & Wallace, 1938). These facts do not seem to support the idea of virus multiplication. On the other hand, Maramorosch (1955) has developed a new technique for the study of this virus and he considers that his results support the idea of multiplication. Using an improved type of micro-injector, he inoculated virus-free leafhoppers with virus-containing juice at dilutions of 1 : 30 and 1 : 300. Insects receiving the lower dilution became infective after 1–9 days, whilst those receiving the higher dilution only became infective after 5–20 days. This suggests a period of incubation or multiplication of the virus in the insect.

Transmission by Mandibulate Insects

The transmission of tobacco mosaic virus by means of the grasshopper, which was briefly discussed under the heading of mechanical transmission, is not again dealt with here. Only those cases of transmission by biting insects are discussed, where it is considered that some sort of biological relationship exists between vector and virus.

There are at least three instances of virus transmission by biting insects which fall into this category; these are turnip yellow mosaic virus, certain squash mosaic viruses and the virus of cowpea mosaic.

In the case of turnip yellow mosaic virus various species of beetles, beetle larvae, grasshoppers and earwigs (Forficulidae) were all found capable of transmitting the virus (Smith & Markham, 1946; Markham & Smith, 1949). All these different types of insects have one characteristic in common, they regurgitate whilst feeding. This seems to be a necessary corollary to transmission since biting insects which do not regurgitate, such as caterpillars, are unable to act as vectors. The virus is retained for a considerable period by the insect, from 10 to 14 days in the case of turnip yellow mosaic virus, 17 to 20 days for squash mosaic virus (Freitag, 1956) and 14 days for cowpea mosaic virus (Dale, 1953). Infective larvae of the mustard beetle (*Phaedon cochleariae*) do not retain the virus of turnip yellow mosaic through pupation, nor does the virus overwinter in hibernating adult beetles (*Phyllotreta* spp.). Squash mosaic virus can be recovered in an infective condition from the blood, faeces and regurgitated gut contents of the cucumber beetles, and in this case the virus may possibly overwinter in the hibernating adults (Freitag, 1956).

It is rather difficult to assess the exact relationship between these viruses and their mandibulate vectors. There is no doubt that more is involved than a purely mechanical relationship since the virus is retained by the insects for considerable periods. It may be suggested that transmission only lasts so long as undigested virus material remains in the foregut and can still be regurgitated. The matter, how-

ever, is probably not as simple as that, especially when, as in the case of squash mosaic, the virus can be detected in the blood. However, since there is little evidence of multiplication of virus in the insect the eventual explanation may turn out to be a build-up of virus concentration in the insect.

It is interesting that though all three beetle-transmitted viruses are also easily sap-inoculable, none can be transmitted by sucking insects such as aphids. The reason for this lies presumably in a property of the viruses since they must be easily accessible to the feeding aphid.

Viruses in Non-vector Insects

It is known in several cases that virus imbibed by or injected into a non-vector insect species does not become immediately inactivated and may in fact persist for long periods. Thus the virus of beet curly-top will persist for 14–21 days in a non-vector species of leafhopper and in an aphid, but neither insect is able to transmit the virus (Bennett & Wallace, 1938). Similarly, the virus of curly-top could be recovered from the faeces of flea beetles, and tobacco mosaic virus after ingestion by caterpillars (Smith, 1941). The virus of turnip yellow mosaic is also resistant to the digestive juices of non-vector insects such as caterpillars and even to the digestive enzymes of snails. There must therefore be other reasons for non-transmission than rapid inactivation in the body of the insect.

Maramorosch (1955) studied the duration of retention of the aster yellows virus by vector and non-vector leafhoppers. He inoculated the juices of the two types of leafhoppers, after feeding on a source of aster yellows virus, at a dilution of 1 : 100, into virus-free aster leafhoppers. He found that it took as long as 66 days for those insects inoculated with the juice of non-vector insects to develop infectivity. Such a long incubation period suggests that the amount of virus was very small. By means of such titrations of the virus recovered from the bodies of the insects it appeared that there was a slow loss of virus from the

non-vector species, whilst the vector species retained the virus for the rest of their lives.

It may be, of course, that in certain cases non-vector species actually do not imbibe the virus whilst feeding. The reason for this is not clear, but some support is given to it by experiments carried out by van Soest & de Meester-Manger Cats (1956) previously mentioned. They were unable to detect the virus of tobacco mosaic in the droplets of plant sap exuding from the cut ends of aphids' stylets *in situ* in mosaic tobacco plants.

Variations in Vector Efficiency

Under this heading are discussed variations in the transmitting power of individual insects belonging to the same vector species.

There seems little doubt that there is individual variation among aphid vectors as regards efficiency of transmission with some viruses and Stubbs (1955) suggests that there may exist races of *Myzus persicae* unable to transmit the virus of spinach yellows of which *M. persicae* is the normal vector. The converse of this has been suggested by Bawden & Kassanis (1947), who think there may occur individual aphids capable of transmitting a virus which is not spread by the species as a whole.

An interesting case of transmission by a particular form of an aphid vector has been reported by Paine & Legg (1953), who found that the virus of hop mosaic is transmitted only by the winged form of *Phorodon humuli* Schrank and not by the wingless form.

Cases of differential transmission by developmental stages of insect vectors are also known. The virus of tomato spotted wilt is transmitted by one or two species of thrips (Thysanoptera), but only the larval form can pick up and transmit this virus. The adult thrips can transmit the virus if it has acquired it during its larval life, but it cannot acquire the virus *de novo* in the adult stage. The reason for this phenomenon is not certainly known, although Bawden (1950) has suggested that the gut wall of the adult insect

may be more impermeable to virus than that of the larva. On this assumption virus picked up during the larval stage of the insect would pass through the gut wall and circulate in the blood, where it remains for the rest of the insect's life, thus allowing the adult to transmit the virus but not to acquire it.

Kunkel (1926) showed that the virus of aster yellows was transmitted by the adult leafhopper but not by the nymph, the reason for this being the fact that the incubation period of the virus in the insect was longer than the nymphal life of the leafhopper. Kunkel demonstrated this by keeping the larvae at low temperatures; this retarded the development of the nymphs but not the development of infective power. Under these conditions the larval leafhopper becomes infective.

The existence of definite races of the same vector species which were respectively able and unable to transmit a given virus was first demonstrated by Storey (1932) working with the streak virus of maize and the leafhopper vector *Cicadulina mbila* Naude. He named these races *active* and *inactive*, according to their transmitting capacity. No difference in the external morphology of the two races could be detected and there is little doubt that both fall into the one species *C. mbila* Naude. Storey also showed that by the crossing of pure races the ability to transmit is inherited as a simple dominant Mendelian factor, linked with sex. In a later paper (1933) Storey gave results of inoculating and puncturing leafhoppers. This was probably the first record of the successful inoculation of a plant virus into an insect vector to render it infective. By this means it was possible to show that in an active infective leafhopper the virus was present in the contents of the rectum if the insect had fed recently on a diseased plant but not otherwise. It was also present in the general contents of the thorax, abdomen and blood, but not in the naturally voided faeces. The virus appeared in the blood before the insect developed infective power. It was possible to render an inactive insect capable of transmitting the virus if a

simple puncture was made in the abdomen. It was necessary, however, for the puncture to be made in some part of the intestine. Inactive races could also be rendered infective by inoculation with the streak virus, although the numbers of successes were significantly less than with active races.

This is probably in no way an isolated phenomenon, but may occur with several of the leafhopper-transmitted viruses. Indeed, Black (1943) has shown that something of the same sort occurs with the New York strain of potato yellow dwarf virus and the leafhopper *Aceratagallia sanguinolenta* Prov. This case, however, is not so clear-cut as the foregoing. In the 'active' races, the virus was transmitted by 80 per cent. of the insects, and by only 2 per cent. in the 'inactive'; whilst of the hybrids 30 per cent. transmitted the virus.

As suggested by Storey, the inability to transmit is probably due to some factor in the intestinal wall which, in the inactive races, resists the passage of the virus and so prevents it reaching the blood and thence the salivary glands.

Kunkel (1954) suggests, as a reason for differences in vector efficiency of the aster leafhopper, that the insects themselves vary in their susceptibility to infection with aster yellows virus. If we accept the suggestion—and the facts now seem to support it—that the infective aster leafhopper is itself diseased, then, of course, efficiency as a vector may depend on a variety of factors. There is the length of life of the transmitting insect, the rate of multiplication of the virus and the concentration inside the insect, and any possible ill effect of the virus itself upon the insect's own metabolism.

EFFECT OF PLANT VIRUSES UPON THE INSECT VECTOR

The question as to whether a plant virus has any effect, deleterious or otherwise, upon the insect vector is one which

has interested virus workers for many years. One of the earliest attempts to investigate the matter was made by Dobroscky (1929), who undertook an intensive study of the salivary glands and alimentary tract of the leafhopper, vector of the aster yellows virus *Macrosteles fascifrons* Stal., with a view to finding any difference between viruliferous and non-viruliferous insects. Dobroscky was unable to find any difference between the two. The writer has carried out a similar study in conjunction with Dr K. Maramorosch, using the electron microscope to examine thin sections of the salivary glands of *M. fascifrons* with aster yellows virus and of *Circulifer* (= *Eutettix*) *tenellus* Baker with beet curly-top virus. Careful examination was also made of similar material from virus-free leafhoppers. These studies were negative and no difference could be discerned between the viruliferous and virus-free material. The reason for this failure now seems apparent in a recent paper by Littau & Maramorosch (1956) who have made a cytological study with the optical microscope of the aster leafhopper (*M. fascifrons* Stal.) They examined not the salivary glands but the fatbody and found a difference between the viruliferous and virus-free insects. This difference was found in the cells. In the virus-free leafhoppers the nuclei tended to be round or to have smooth contours; only a few were stellate and these were observed in only 36 per cent. of the leafhoppers. The cytoplasm was generally homogeneous with a large number of vacuoles of varying size and the cells were intact. In viruliferous insects almost all nuclei of the fatbody cells were stellate (in 95 per cent. of the leafhoppers) and the cytoplasm was reticulate. Many cells seemed abnormal and appeared broken in the sections.

Littau & Maramorosch consider that this is not merely a case of an insect vector of a virus but rather that the insect is an infected host of the aster yellows virus. They state that the persistent transmission of the virus may be a result of progressive deterioration of fatbody cells in which the virus is stored and from which it is being released into the blood. It is rather interesting to consider that the fatbody is the

site of multiplication of at least three types of insect viruses, but these involve destruction of the fatbody and rapid death of the infected larva.

A slightly different effect of a plant virus on its insect vector may appropriately be considered here. It has long been known that a type of non-sterile immunity exists between plant viruses and related strains. In other words, it is usually not possible to inoculate a virus into a plant already infected with a related virus. The classical example of this is the inability of a 'yellow mottle' strain of tobacco mosaic virus to infect a tobacco plant already infected with a 'green mottle' strain of the same virus. Two strains of aster yellows virus exist, known respectively as aster yellows virus and Californian aster yellows virus. Until recently it has not been possible to test whether a cross-immunity existed between these two viruses because the symptoms produced on the test plants were indistinguishable. Now, however, Kunkel (1955) has found two test plants which react in a different manner to the two viruses. The plants are *Vinca rosea* and *Nicotiana rustica* L. This enabled cross-immunity tests to be carried out, which showed that the aster yellows virus protected against the Californian aster yellows virus, and vice versa. The discovery of these two differential hosts also enabled Kunkel to carry out experiments designed to show whether a similar cross-immunity existed in the insect vector. Virus-free leafhoppers were fed first on a plant infected with aster yellows virus and then on a plant infected with Californian aster yellows virus. When transferred to healthy plants only the aster yellows virus was transmitted, and when the procedure was reversed the plants developed only the Californian aster yellows disease. Kunkel points out that these experiments prove only that leafhoppers, infective for one virus, do not transmit the other. They have not shown that both viruses may not be picked up by the insects. Presumably, however, the virus first acquired is multiplying inside the insect and so the available multiplication sites are occupied, leaving no opportunity for the second virus to reproduce itself.

This seems to be the only case, so far known, of cross-protection of plant viruses in an insect vector. The position seems very different with the aphid-borne viruses; so far as the writer is aware, there is nothing to prevent an aphid vector acquiring consecutively and transmitting any number of allied viruses, such as the different strains of cucumber mosaic virus.

LOCATION AND VISUALIZATION OF VIRUS IN THE VECTOR

The development of the technique of cutting ultrathin sections for the electron microscope has opened up possibilities of locating and observing plant viruses in the insect vector. So far little seems to have been done in this direction other than some preliminary work by the writer which, as previously pointed out, was entirely negative, probably because the wrong organs were examined. The work of Littau & Maramorosch (1956) suggests that the fatbody rather than the salivary glands is the site of virus multiplication, and examination of this by means of thin sections on the electron microscope should prove fruitful.

FEEDING HABITS, ENVIRONMENTAL CONDITIONS AND HOST PLANT SPECIES IN RELATION TO VIRUS TRANSMISSION

In this section are discussed some of the many variable factors which influence the spread of insect-borne plant viruses in the field.

In some cases the intrinsic properties of the virus itself may profoundly affect its spread by the interplay of insect relationships and the weather. Thus, if a virus is of the persistent type, such as that of potato leaf-roll, it can be carried long distances by aphids drifting on the prevailing winds. Aphids can acquire and transmit leaf-roll virus only after feeding periods of some hours, and so spread within the crop is favoured by conditions that restrict frequency of

flight. On the other hand, potato virus Y is a non-persistent virus and does not remain long infective in the aphid vector, and it spreads mostly in calm, warm weather suitable for flight, particularly early in the season when aphids are colonizing crops (Broadbent, 1953).

Because of the low thermal inactivation point of the aster yellows virus, climate and season have a profound effect upon the ability of the leafhopper *Macrosteles fascifrons* to transmit it. Infective insects that are exposed to high temperatures such as frequently prevail during the summer months in the U.S.A., especially in the south, lost ability to transmit either temporarily or permanently, depending on the length of the hot spell (Kunkel, 1954).

Another combination of circumstances which affects the spread of aphid-borne viruses is the concentration of the virus or viruses in certain leaves and the availability of such leaves to alighting aphids.

There are two common viruses affecting cauliflowers, the cabbage black ringspot and cauliflower mosaic viruses. Both are non-persistent viruses transmitted by the aphid *Myzus persicae* Sulz., yet cauliflower mosaic virus is much more common in fields of cauliflower than is cabbage black ringspot virus which occurs in higher concentration and has a much wider host range than the former. Broadbent (1954) suggests that at least part of the difference between the rates at which the two viruses spread in the field may be accounted for by the different manner in which they are distributed in old infected plants, and the effect this has on transmission by aphids. Cauliflower mosaic virus occurs in high concentration in all the new leaves produced by infected plants. Cabbage black ringspot virus, on the other hand, occurs mainly in the older leaves, and even there is localized in parts that show symptoms. After flying, most aphids alight on the upper parts of plants; they are therefore less likely to acquire cabbage black ringspot virus than cauliflower mosaic virus. The distribution of viruses in different leaf tissues and its influence on virus transmission by aphids has been studied by Bawden, Hamlyn & Watson

(1954). These workers consider that some of the anomalies in aphid transmission can be explained by the unequal distribution of readily extractable virus in different tissues of systemically infected leaves and its relatively high concentration in epidermal cells.

In some cases it appears that the aphid can acquire the virus from one tissue in the plant, but to secure infection the virus must be injected into another. The beet yellow-net virus can be acquired by the aphid in 5 minutes, but the inoculation threshold is approximately 15 minutes after feeding commences. Sylvester (1949*a*) interprets this on the assumption that mesophyll penetration is sufficient to acquire the virus, but the phloem is the essential tissue involved in inoculation.

Although the species of plant which acts as the virus source is not known to affect vector efficiency, the species of plant inoculated by the aphid may have some bearing on the matter. Thus in the case of *Brassica nigra* virus, *Myzus persicae* was a more efficient vector to mustard, but *Rhopalosiphum pseudobrassicae* was a better vector when transmission was made to Chinese cabbage (*Brassica chinensis* L.) (Sylvester & Simons, 1951).

The behaviour of aphid vectors depends a good deal on the weather, and more winged individuals (*alatae*) develop in the south of the British Isles than in the north (Broadbent, 1953).

Aphids do not sense and make their way towards a favoured crop and, in any case, they move largely by drift since they cannot make any headway against a wind. The alighting response seems to be a non-specific visual one evoked by objects looming up in the path of the flying insects. For example, in 1947, autumn migrants of the peach-potato aphid, *Myzus persicae*, were seen alighting quite indiscriminately on their specific winter host, the peach, and on another tree, spindle, on which they do not overwinter. Thus the aphids exercised their selection between suitable and unsuitable hosts mainly after alighting on them. From this it seems clear that winged migrants do

visit and feed on plants which they do not colonize. As the result of these observations Kennedy (1950) suggests that the key considerations affecting the virus-spreading efficiency of a given aphid species are its ability to transmit the virus and the abundance and activity of its winged forms, rather than its potentialities as a direct pest of the crop. Thus among the winged aphids available and capable of transmitting a virus those species also capable of becoming serious pests would be at some disadvantage as virus spreaders compared with species less well adapted to the given plants. In other words, the casual winged insect vector which may alight and feed on a plant before moving off again is a more important agent in the spread of a virus than the vector which alights and remains to colonize the crops.

The feeding habits of an insect may play an important part in determining its role as a vector. For example, the virus of alfalfa dwarf (Pierce's disease of the grape) is transmitted by a number of leafhoppers, all of which belong to the subfamily Tettigellinae. All these insects have one characteristic in common—they are without exception xylem feeders. If they are prevented mechanically from reaching the xylem then they cannot transmit the virus (Houston, Esau & Hewitt, 1947). Two other examples of this occur with the vectors of peach phony disease (Turner, 1949) and chlorotic streak of sugar cane (Abbot & Ingram, 1942).

On the other hand, the leafhopper vector of beet curly-top is a phloem feeder and cannot either transmit the virus or survive if it is unable to reach the phloem (Bennett, 1934). Sometimes the exact area of an infected leaf selected by a potential vector is important. Storey (1938) has shown that the leafhopper *Cicadulina mbila* cannot take up the maize streak virus by feeding on the green areas of the leaf which separate the chlorotic areas induced by the disease.

TRANSMISSION OF PLANT VIRUS COMPLEXES

Three types of differential transmission by aphid vectors of virus complexes are considered here. The first is a straight-

forward selective transmission; the second is also selective but is contingent upon the latent period of a virus in the insect. In the third type, aphid transmission of a virus is dependent upon the presence in the plant of a second virus.

The first type of differential transmission by aphids of a virus complex can be subdivided into two. Thus, two viruses may occur together in a plant only one of which is aphid-borne. Several examples of this are known, the commonest being the combination of potato virus X, which is not aphid-transmitted, with potato viruses Y or A which are. Here, of course, the insect virus selects out the virus which it can transmit, leaving the other virus behind. In the other case, a plant may be infected with two aphid-borne viruses and the selective transmission depends upon the aphid species. For example, when the aphids *Myzus persicae* and *Brevicoryne brassicae* L. are colonized upon cauliflower seedlings infected with the cabbage black ringspot and cauliflower mosaic viruses they are able to transmit both, but the aphid *Myzus ornatus* Laing, similarly colonized, picks out the cauliflower mosaic virus, leaving the black ringspot virus behind (Kvicala, 1945). The aphid *M. ascalonicus* Donc. will transmit the viruses of cucumber mosaic and henbane mosaic, but not potato virus Y and severe etch virus. This aphid, therefore, will select out cucumber mosaic virus from a plant infected with a mixture of this and potato virus Y. In the same way, when fed on leaves containing henbane mosaic and severe etch viruses it transmits only henbane mosaic (Doncaster & Kassanis, 1946).

In discussing the aphid-transmitted persistent viruses we have mentioned the long latent period exhibited by 'Virus 3' of strawberries in the aphid vector *Capitophorus fragariae* Theob., in which 10–19 days elapse before the insect becomes infective. This virus is one component of the strawberry disease known as 'severe crinkle' and Prentice (1949) isolated it by means of the aphid *C. fragariae* which was allowed to feed for several days on a strawberry plant infected with severe crinkle. The aphids picked up the two viruses, the second one being the strawberry mottle virus;

this, being of the non-persistent type, was eliminated by transferring the aphids to fresh indicator plants after 24 hours. Thus, by taking advantage of the differences in the times the 'persistent' and 'non-persistent' viruses remain in the aphid vector it is possible to separate out a virus complex.

We turn now to the third type of relationship of aphids with plant virus complexes in which one virus is dependent upon another for aphid transmission. The best known example of this phenomenon is the rosette disease of tobacco (Smith, 1946), which is caused by two viruses, the vein-distorting and mottle viruses, respectively. Both these viruses when together in the plant are of the persistent type and are transmitted with great efficiency by the aphid *Myzus persicae* Sulz.; when separated only the vein-distorting virus is aphid-borne. It appears to be necessary for both viruses to be together in the plant to enable the aphid to pick up the mottle virus; it is not sufficient for the aphid to feed first on a plant with vein-distorting virus and then on a plant with mottle virus. Quite a lot of investigation has been made of this problem, but the reason for it is still obscure. The obvious explanation that in the presence of the vein-distorting virus the mottle virus occurs in higher concentration does not seem to be the answer. Another example of the same phenomenon has been observed by Clinch, Loughnane & Murphy (1936), who state that it is necessary for potato virus A to be present in the potato plant to enable the aphid *Myzus persicae* to pick up potato virus F (tuber blotch virus), but no experimental data are given.

Transmission by Vectors other than Insects

There are at least four cases of plant virus transmission by mites (Acarina), and now that this type of vector has been recognized no doubt others will be discovered. The first record was that of the reversion disease of black currants transmitted by the big bud mite, *Phytoptus ribis* (Westw.) Nalepa (Amos, Hatton, Knight & Massee, 1927; Massee,

1952). Then Slykhuis (1955) demonstrated that the Eriophyid mite *Aceria tulipae* K. was the vector of the virus of wheat streak mosaic and Flock & Wallace (1955) have shown that the virus of fig mosaic is transmitted by the mite *A. ficus* Cotte. Finally, the long-sought-for vector of the American peach mosaic virus has now been identified as a mite (Cochran, Jones & Wilson, 1955).

Not much is known of the relationship between this type of vector and the viruses transmitted. Slykhuis (1955) has shown that when the mite *Aceria tulipae* was reared on wheat infected with streak mosaic all stages except the eggs carried the virus. However, it was found that when virus-free mites in different developmental stages were colonized on diseased wheat the nymphs could acquire the virus but the adults could not. This is a similar phenomenon to that which occurs in the transmission of tomato spotted wilt virus by thrips where the adult insect cannot pick up the virus *de novo*.

Hewitt *et al.* (1958) have demonstrated that the virus of grape fanleaf is transmitted by a species of nematode worm, *Xiphinema index* Thorne & Allen. Healthy grape growing with a fanleaf-diseased grape in the same clay pot developed fanleaf within 10 months of being planted and infested with 10 *X. index* adults from the root zone of healthy grape. Similarly, fanleaf developed in healthy grape growing with fanleaf-diseased grape in soil infested with *X. index* from the root zone of fig. In another experiment healthy grape, growing in pasteurized soil, free from eelworm in a nursery container with a fanleaf-diseased grape, remained healthy for 3 years.

This is the first record of plant virus transmission by an eelworm, but it probably will not be the last.

DISCUSSION

It can be seen from the foregoing account that a great many facts relating to insects and plant viruses have now been

assembled, but the situation is very far from clear. It seems well established, however, that certain plant viruses do multiply in their leafhopper vectors.

It is not known how far the transmission of viruses by other vectors, especially aphids, is a fixed biological relationship. In other words, does the virus mutate so that it becomes insect-borne or can vectors become 'attuned' to transmit plant viruses in a comparatively short time? Viruses which have no known insect vector but have alternative methods of spread, as in the case of tobacco mosaic virus and potato virus X, keep going. On the other hand, it can be suggested that plant viruses may appear and then, if no insect vector or alternative method of spread develop, the viruses die out unless propagated artificially in laboratories. An example of this is lovage mosaic which failed to pass to neighbouring lovage plants over a period of 10 years and indeed was almost impossible to transmit artificially to healthy lovage plants, although it could be passed easily enough by mechanical inoculation to other miscellaneous hosts. The virus of tomato bushy stunt presents an interesting case of the disappearance of a plant virus in the apparent absence of an insect vector. It was first described in England by Smith in 1935, and except for one brief appearance has not been seen since. Now it has turned up again in Italy, where it has been found naturally infecting *Petunia*. This is important because it would appear that an insect vector (possibly of the mandibulate type) for the virus must have developed. This seems to be the only explanation of how the virus gets to *Petunia* unless it is soil-transmitted.

Viruses long prevented artificially from having contact with their insect vectors tend to lose their insect-transmissibility (Black, 1953). Is the converse true? If insects, or other arthropods, are bred continuously on virus-infected plants, will they eventually become vectors? Why should the virus of turnip yellow mosaic, which is highly infectious, only be transmitted by biting insects, especially the turnip flea beetle, and not by aphids? Why should the virus of fig

mosaic be transmitted only by mites? Can the explanation lie in the long association of these arthropods with these two hosts? Fig trees in California are almost universally infected with the fig mosaic virus.

On the other hand, it may happen that an insect which casually visits a virus-infected plant becomes a vector rather than the more common insect fauna of that plant. This would account for sudden outbreaks of new virus diseases. The insect transmission of peach phony disease and sandal spike seem to be cases in point.

It does not seem possible to correlate physical or other properties of viruses with insect relationships, although it is true that many aphid-borne viruses are unstable and occur in low concentration in the host plant.

It may be that in the past too much attention has been paid to the well-known insect fauna of virus-infected plants and not enough to more obscure organisms. Now that consideration has been given to mites as vectors we already have four authentic cases of mite-transmitted plant viruses. There may be other organisms, notably in the soil, which could act as vectors; we have already seen that nematodes can transmit the virus of grape fanleaf, or some entirely unsuspected mode of spread may exist. Transmission by the soil needs more investigation; the tobacco necrosis viruses are soil-borne and their method of spread is more like that of fungal or bacterial spores than of a plant virus. The virus of wheat rosette or mosaic is known to be soil-borne, but the mechanics of its spread are not understood.

REFERENCES

ABBOTT, E. G. and INGRAM, J. W. (1942). 'Transmission of chlorotic streak of sugar cane by the leafhopper *Draeculacephala portola*.' *Phytopathology*, **32**, 99.

ALLARD, H. A. (1914). 'The mosaic disease of tobacco.' *U.S. Dept. Agric. Bull.* no. 40.

AMOS, J., HATTON, R. G., KNIGHT, R. C. and MASSEE, A. M. (1927). 'Experiments in the transmission of reversion

in black currant.' *Ann. Rep. E. Malling Res. Sta.* **11,** 126.

BALL, E. D. (1909). 'The leafhoppers of the sugar beet and their relation to the curly-top condition.' *Bull. U.S. Dept. Agric. Ent.* **66,** 33.

BAWDEN, F. C. (1950). *Plant Viruses and Virus Diseases.* 3rd ed. Waltham, Mass.; Chronica Botanica Co.

BAWDEN, F. C., HAMLYN, BRENDA M. G. and WATSON, A. MARION (1954). 'The distribution of viruses in different leaf tissues and its influence on virus transmission by aphids.' *Ann. appl. Biol.* **41,** 229.

BAWDEN, F. C. and KASSANIS, B. (1947). 'The behaviour of some naturally occurring strains of potato virus Y.' *Ann. appl. Biol.* **34,** 503.

BENNETT, C. W. (1934). 'Plant-tissue relations of the sugar beet curly-top virus.' *J. agric. Res.* **48,** 665.

BENNETT, C. W. and WALLACE, H. E. (1938). 'Relation of the curly-top virus to the vector, *Eutettix tenellus*.' *J. agric. Res.* **56,** 31.

BLACK, L. M. (1940). 'Mechanical transmission of aster yellows virus to leafhoppers.' *Phytopathology,* **30,** 2.

(1941). 'Further evidence for multiplication of the aster yellows virus in the aster leafhopper.' *Phytopathology,* **31,** 120.

(1943). 'Genetic variation in the clover leafhopper's ability to transmit potato yellow dwarf virus.' *Genetics,* **28,** 200.

(1950). 'A plant virus that multiplies in its insect vector.' *Nature, Lond.* **166,** 852.

(1953). 'Loss of vector transmissibility by viruses normally insect-transmitted.' *Phytopathology,* **43,** 466.

BRADLEY, R. H. E. (1952). 'Studies on the aphid transmission of a strain of henbane mosaic.' *Ann. appl. Biol.* **39,** 78.

BROADBENT, L. (1953). 'Aphids and virus diseases in potato crops.' *Biol. Rev.* **28,** 350.

(1954). 'The different distribution of two brassica viruses

in the plant and its influence on spread in the field.' *Ann. appl. Biol.* **41**, 174.

CADMAN, C. H. and HARRISON, B. D. (1956). 'Transmission of leaf-roll virus.' *3rd Ann. Rep. Scot. Hort. Res. Inst.* p. 23.

CLINCH, P., LOUGHNANE, J. B. and MURPHY, P. (1936). 'A study of the aucuba or yellow mosaics of the potato.' *Sci. Proc. Roy. Dublin Soc.* **21**, 431.

COCHRAN, L. C., JONES, L. S. and WILSON, N. S. (1955). 'Mite vector of peach mosaic.' *Agric. Res. U.S.D.A.*, 15 Sept.

DALE, W. T. (1953). 'The transmission of plant viruses by biting insects with particular reference to cowpea mosaic.' *Ann. appl. Biol.* **40**, 384.

DAY, M. F. (1955). 'Mechanism of transmission of potato leaf-roll by aphids.' *Austral. J. Biol. Sci.* **8**, 498.

DAY, M. F. and IRZYKIEWICZ, H. (1954). 'On the mechanism of transmission of non-persistent phytopathogenic viruses by aphids.' *Austral. J. Biol. Sci.* **7**, 251.

DOBROSCKY, I. B. (1929). 'Is the aster yellows virus detectable in its insect vector?' *Phytopathology*, **19**, 11.

DONCASTER, J. P. and KASSANIS, B. (1946). 'The shallot aphis, *Myzus ascalonicus*, Donc., and its behaviour as a vector of plant viruses.' *Ann. appl. Biol.* **33**, 66.

DOOLITTLE, S. P. (1916). 'A new infectious disease of cucumber.' *Phytopathology*, **6**, 145.

DOOLITTLE, S. P. and GILBERT, W. W. (1918). 'Further notes on cucumber mosaic disease.' *Phytopathology*, **8**, 77.

DOOLITTLE, S. P. and WALKER, M. N. (1928). 'Aphis transmission of cucumber mosaic.' *Phytopathology*, **18**, 143.

FLOCK, R. A. and WALLACE, J. M. (1955). 'Transmission of fig mosaic by the Eriphyid mite *Aceria ficus*.' *Phytopathology*, **45**, 52.

FREITAG, J. H. (1956). 'Beetle transmission, host range and properties of squash mosaic virus.' *Phytopathology*, **46**, 73.

FUKUSHI, T. (1933). 'Transmission of a virus through the eggs of an insect vector.' *Proc. imp. Acad. Japan*, **9**, 451.

HEWITT, W. B., RASKI, D. J. and GOHEEN, A. C. (1958). 'Transmission of fanleaf virus by *Xiphinema index*, Thorne and Allen.' *Phytopathology* (abstr.), **48**, 393–4.

HOGGAN, I. A. (1933). 'Some factors involved in aphid transmission of the cucumber mosaic virus to tobacco.' *J. agric. Res.* **47**, 689.

HOUSTON, B. R., ESAU, KATHERINE and HEWITT, W. B. (1947). 'The mode of vector feeding and the tissues involved in the transmission of Pierce's disease virus in grape and alfalfa.' *Phytopathology*, **37**, 247.

JAGGER, I. E. (1916). 'Experiments with the cucumber mosaic disease.' *Phytopathology*, **6**, 148.

KASSANIS, B. (1952). 'Some factors affecting the transmission of leaf-roll virus by aphids.' *Ann. appl. Biol.* **39**, 157.

KENNEDY, J. S. (1950). 'Aphid migration and the spread of plant viruses.' *Nature, Lond.* **165**, 1024.

KIRKPATRICK, H. C. and ROSS, A. F. (1952). 'Aphid transmission of potato leaf-roll virus to Solanaceous hosts.' *Phytopathology*, **42**, 540.

KLOSTERMEYER, E. C. (1953). 'Entomological aspects of the potato leaf-roll problem in central Washington.' *Wash. agric. Exp. Sta. Tech. Bull.* no. 9, p. 1.

KUNKEL, L. O. (1926). 'Studies on aster yellows.' *Amer. J. Bot.* **13**, 646.

(1937). 'Effect of heat on ability of *Cicadula sexnotata* to transmit aster yellows.' *Amer. J. Bot.* **24**, 316.

(1948). 'Studies on a new corn virus disease.' *Arch. ges. Virusforsch.* **4**, 24.

(1954). 'Maintenance of yellows-type viruses in plant and insect reservoirs.' *Dynamics of Virus and Rickettsial Infections, International Symposium*, p. 209. New York: Blakiston Co.

(1955). 'Cross-protection between strains of yellows-type viruses.' *Advances in Virus Research*, **3**, 251.

KVICALA, B. (1945). 'Selective power in virus transmission exhibited by an aphis.' *Nature, Lond.* **155,** 174.

LITTAU, V. C. and MARAMOROSCH, K. (1956). 'Cytological effects of aster yellows virus on its insect vector.' *Virology*, **2,** 128.

MARAMOROSCH, K. (1951). 'Mechanical transmission of corn stunt virus to an insect vector.' *Phytopathology* (abstr.), **41,** 658.

(1952). 'Direct evidence for the multiplication of aster yellows virus in its insect vector.' *Phytopathology*, **42,** 59.

(1955). 'Multiplication of plant virus in insect vectors.' *Advances in Virus Research*, **3,** 221.

(1956). 'Multiplication of aster yellows virus in *in vitro* preparations of insect tissues.' *Virology*, **2,** 369.

MARKHAM, R. and SMITH, K. M. (1949). 'Studies on the virus of turnip yellow mosaic.' *Parasitology*, **39,** 330.

MASSEE, A. M. (1952). 'Transmission of reversion of black currants.' *Ann. Rep. E. Malling Res. Sta.* (1951), p. 162.

MERRILL, H. M. and TENBROECK, C. (1934). 'Multiplication of equine encephalomyelitis in mosquitoes.' *Proc. Soc. exp. Biol. N.Y.* **32,** 421.

PAINE, J. and LEGG, J. T. (1953). 'Transmission of hop mosaic by *Phorodon humuli*, Schrank.' *Nature, Lond.* **171,** 263.

PRENTICE, I. W. (1949). 'Resolution of Strawberry Virus complexes. III. The isolation and some properties of Virus 3.' *Ann. appl. Biol.* **36,** 18.

SHAW, H. B. (1910). 'The curly-top of beets.' *U.S. Dept. Agric. Bur. Pl. Ind. Bull.* no. 181.

SLYKHUIS, J. T. (1955). '*Aceria tulipae* Keifer (Acarina: Eriphyidae) in relation to the spread of wheat streak mosaic.' *Phytopathology*, **45,** 116.

SMITH, K. M. (1935). 'A new virus disease of the tomato.' *Ann. appl. Biol.* **22,** 731.

(1941). 'Some notes on the relationship of plant viruses

with vector and non-vector insects.' *Parasitology*, **33**, 110.

(1946). 'The transmission of a plant virus complex by aphids.' *Parasitology*, **37**, 131.

SMITH, K. M. and LEA, D. E. (1946). 'The transmission of plant viruses by aphids.' *Parasitology*, **37**, 25.

SMITH, K. M. and MARKHAM, R. (1946). 'An insect vector of the turnip yellow mosaic virus.' *Nature, Lond.* **158**, 417.

SOEST, W. VAN and MEESTER-MANGER CATS, V. DE (1956). 'Does the aphid *Myzus persicae* (Sulz.) imbibe tobacco mosaic virus?' *Virology*, **2**, 411.

STOREY, H. H. (1932). 'The inheritance by an insect vector of the ability to transmit a plant virus.' *Proc. roy. Soc. B*, **112**, 46.

(1933). 'Investigation of the mechanism of the transmission of plant viruses. I.' *Proc. roy. Soc. B*, **113**, 463.

(1938). 'Investigation of the mechanism of the transmission of plant viruses by insect vectors. II. The part played by puncture in transmission.' *Proc. roy. Soc. B*, **125**, 455.

STUBBS, L. L. (1955). 'Strains of *Myzus persicae* Sulz. active and inactive with respect to virus transmission.' *Austral. J. Biol. Sci.* **8**, 68.

SUKHOV, K. S. (1944). 'Salivary secretion of the aphid *Myzus persicae* (Sulz.) and its ability to form a filtering apparatus.' (In Russian.) *C.R. Acad. Sci. U.R.S.S.* **42**, 226.

SYLVESTER, E. S. (1949*a*). 'Transmission of sugar beet yellow-net virus by the green peach aphid.' *Phytopathology*, **39**, 117.

(1949*b*). 'Beet mosaic virus-green peach aphid relationships.' *Phytopathology*, **39**, 417.

(1954). 'Aphid transmission of non-persistent plant viruses with special reference to the *Brassica nigra* virus.' *Hilgardia*, **23**, 53.

SYLVESTER, E. S. and SIMONS, J. N. (1951). 'Relation of plant species inoculated to efficiency of aphids in the

transmission of *Brassica nigra* virus.' *Phytopathology*, **41**, 908.

TAKATA, K. (1895). 'Results of experiments with dwarf diseases of the rice plant.' *J. Japan. Agric.* **171**, 1.

TRAGER, W. (1938). 'Multiplication of the virus of equine encephalomyelitis in surviving mosquito tissues.' *Amer. J. trop. Med.* **18**, 387.

TURNER, W. F. (1949). 'Insect vectors of phony peach disease.' *Science*, **109**, 87.

WALTERS, H. J. (1952). 'Some relationships of three plant viruses to the differential grasshopper *Melanoplus differentialis* (Thos.).' *Phytopathology*, **42**, 355.

WATSON, M. A. (1936). 'Factors affecting the amount of infection obtained by aphis transmission of the virus Hy. III.' *Phil. Trans. B.* **226**, 457.

(1938). 'Further studies on the relationship between *Hyoscyamus* virus 3 and the aphis *Myzus persicae* (Sulz.) with special reference to the effects of fasting.' *Proc. roy. Soc.* B., **125**, 144–70.

WATSON, M. A. and ROBERTS, F. M. (1939). 'A comparative study of the transmission of *Hyoscyamus* virus 3, potato virus Y and cucumber virus I by the vectors *Myzus persicae* (Sulz.), *M. circumflexus* Buckt., and *Macrosiphum gei* (Koch.).' *Proc. roy. Soc. B*, **127**, 543.

(1940). 'Evidence against the hypothesis that certain plant viruses are transmitted mechanically by aphids.' *Ann. appl. Biol.* **27**, 227.

CHAPTER V

Physiology of Plant Virus Diseases

METABOLISM OF VIRUS-INFECTED PLANTS

Respiration

During the last forty years or so a great deal of work has been done on the respiration of virus-diseased plants, much of it with tobacco mosaic virus. The results, however, have been confusing and often contradictory, chiefly because there are so many variable factors not taken into account by the earlier workers. Owen (1955*b*) points out that the variability in respiration rate between comparable leaves of similarly treated plants is so great, even when plants are selected for uniformity of size and appearance, that many replications are necessary to establish unequivocally the nature and magnitude of the effect of infection. Owen's experiments on the respiration rates of mosaic-infected tobacco leaves show that these rates can be higher or lower than, or identical with, those of healthy leaves depending upon (*a*) the time after inoculation; (*b*) the physiological state of the plants; (*c*) the environmental conditions during growth; (*d*) the leaves chosen; or (*e*) the mode of expression of the results. In future work on respiration rates of virus-diseased plants it will be necessary to take these facts into consideration and also to apply adequate statistical tests of significance to any differences obtained.

The effect of infection with tobacco mosaic virus on the respiration rates of detached tobacco leaves in the period immediately after inoculation differed in plants grown at different times of the year. During winter, infection increased respiration rates and in summer decreased them.

In winter-grown plants increasing the light intensity during the period before inoculation decreased respiration rates after infection. Respiration rates began to change in less than one hour after inoculation and are unlikely to be associated with the formation of new virus.

These variations and contradictions are equally conspicuous when plants systemically infected with tobacco mosaic virus are studied. Thus, the rate of CO_2 production per gramme of dry matter of the younger leaves of tobacco plants systemically infected with tobacco mosaic virus was about 10 per cent. less than that of comparable healthy leaves. Older infected leaves, with well-developed mosaic symptoms, had the same respiration rate as comparable healthy leaves. The effects of the virus on the water content were so great that the rate of CO_2 production per gramme fresh weight was sometimes significantly increased by infection (Owen, 1955*a* and *b*, 1956).

On the other hand, another virus may affect the respiration of the same plant in quite a different way. For example, unlike tobacco mosaic virus, which increases the respiration of tobacco leaves within an hour of being inoculated, the virus of tobacco severe etch did not change the respiration rates until the leaves showed external symptoms. The respiration rates of inoculated or systemically infected leaves with symptoms rose to 40 per cent. above that of healthy leaves, three times the increase produced by tobacco mosaic virus. Moreover, the increased respiration rate occurred at all times of the year and was maintained throughout the life of the leaves (Owen, 1957).

In the case of potato leaf-roll, the respiration rates are higher in the diseased than in the healthy plant. According to Whitehead (1934), except for a short period covering the end of dormancy of the tuber to the first unfolding of the leaves, the leaf-roll-infected potato plant respires at a much higher rate than does the healthy one. He concluded that the virus affects the respiration rate not directly, but only by interfering with the translocation of the respirable substrate.

Effect of Virus Infection upon the Chlorophyll

There seems to be some difference of opinion as to whether virus infection destroys the chlorophyll or inhibits its formation. Sheffield (1933) considered that the virus of tomato aucuba mosaic did not affect the chloroplasts in leaves fully developed at the time of infection but did prevent the formation of plastids in young growing leaves. On the other hand, some viruses such as those of cucumber mosaic and tomato stripe do produce chlorosis when rubbed over mature leaves (Smith, 1935). It is possible that both may be true according to the virus concerned. Cook (1947) considers that the virus competes with the plastids for some of the products necessary for their existence, such as phosphorus and nitrogen, but does not destroy them. It is probable, however, that the pathological effect is more complicated than Cook suggests. In the case of potato aucuba mosaic, Clinch (1932) considers that the yellow spots are mainly due to the loss of green pigment, to excessive quantities of starch in the plastids and to alterations in the structure of the chloroplasts which frequently disintegrate.

TRANSLOCATION OR MOVEMENT OF THE VIRUS IN THE PLANT

The study of the movement, or translocation, of viruses in plants can be approached from several viewpoints. There is, first, the *type* of tissue in which the virus moves, secondly, *rate* and *direction* of movement, and, thirdly, the mechanism involved in the movement.

As regards the type of tissue involved, this depends a good deal upon the virus concerned. These tissue relationships seem to be of three kinds.

(1) A relation in which virus is more or less restricted to parenchyma.
(2) A relation in which virus is more or less restricted to the phloem.
(3) A relation in which virus occurs extensively in both phloem and parenchyma (Bennett, 1940).

(4) To these may be added a fourth type where the virus is apparently confined to the xylem. The virus causing Pierce's disease of grapes, now known to be the same as that of alfalfa dwarf disease, is transmitted only by leafhoppers which feed in the xylem. If the insects are prevented mechanically from reaching the xylem, infection does not occur (Houston, Esau & Hewitt, 1947).

Viruses confined to the parenchyma would obviously be greatly handicapped in their movement through the plant, and it is probably only in local lesions formed by some viruses on certain plants that this relationship holds good. Of viruses confined to the phloem, those of curly-top of sugar beet and raspberry leaf-curl (American) have been most studied. Bennett (1927) has shown that these viruses may be confined to certain parts of an infected plant by destroying the phloem connexions between the inoculated portion and other parts of the plant at the time of inoculation. Caldwell (1930) carried out a similar experiment with tomato plants and the virus of tomato mosaic. A 'bridge' was made in the stem by steam, so that only the xylem vessels were left; it was found that when inoculation was made the virus remained in the half of the plant inoculated and was unable to pass the xylem 'bridge'. Caldwell (1934) also claimed that when virus was injected into the xylem vessels, it could not escape therefrom unless the vessels were mechanically injured, whereupon the leaves developed symptoms.

Another virus like those of beet curly-top and raspberry leaf-curl (American) which is possibly confined to the phloem is that known as the tobacco vein-distorting virus (Smith, 1946). Such viruses are rarely sap-transmissible but rely upon an insect vector to inject them directly into the phloem.

Those viruses which occur in both parenchyma and phloem are of the mosaic type, and the best known example of these is the tobacco mosaic virus. The breaking of a leaf

hair with a virus-contaminated instrument is sufficient to allow virus to enter an epidermal cell. The movement at first is slow, the virus passing from cell to cell until it reaches a vein, after which movement becomes more rapid.

The *rate* of movement depends to some extent upon the virus and also upon the kind of plant infected. Thus the virus of beet curly-top moves at a much greater speed in sugar-beet than in tobacco. The measured rates of virus movement following introduction into the plant vary from one-tenth of a centimetre per hour for the virus of tomato mosaic in tomato, to 152·4 centimetres per hour for the virus of curly-top in sugar-beet (Bennett, 1940).

As regards *direction* of movement, it has been shown by Kunkel (1939) that the virus of tobacco mosaic in tomato can move in two directions. His data show that, on reaching the stem, virus frequently travelled both upward and downward, but also frequently travelled downward only and occasionally upward only. This brings us to the question of the *mechanism* of virus movement in the plant. It seems clear that two kinds of movement must be visualized. There is first the slow cell-to-cell movement via the connecting protoplasmic bridges or plasmodesms; such a movement presumably takes place following the infection, for example, of a trichome with tobacco mosaic virus. As regards the cell-to-cell movement of virus via the plasmodesms, a recent paper by Kassanis, Tinsley & Quak (1958) is relevant. Whilst not denying the role of the plasmodesmata in virus movement, they suggest that viruses may move from cell to cell by some other means. This conclusion is based on the results of tissue culture work with tobacco mosaic virus; they found that the virus moved from cell to cell just as easily in their tissue cultures where plasmodesmata do not occur as in leaf tissue where they do.

The second kind of movement is the more rapid one via the phloem. In the first movement the virus is presumably carried round the cell by diffusion and protoplasmic streaming, passing via the plasmodesms or by holes in the cell wall from cell to cell. It seems clear that viruses cannot

pass through the cell wall by diffusion. In the more rapid movement in the phloem these forces presumably play no part, but viruses have been shown to move rapidly in directions of food utilization and storage and slowly in opposite directions. Bennett (1940) considers that in the light of present knowledge it seems probable that the mechanism responsible for virus transport in the phloem is able to effect movements essentially similar to those that would be expected to result if a pressure-flow mechanism such as that proposed by Münch (1930) were operating in the transport of elaborated food materials.

INTERFERENCE BETWEEN VIRUSES

It is a commonplace now in plant virus research that plants, and this includes woody plants like stone fruit trees, are frequently infected with a complex of viruses rather than with one virus alone. From this has arisen a realization of the somewhat involved interaction which may take place when two or more viruses are acting together in the same host. This subject has been reviewed recently by Bennett (1953), to whom the reader is referred for a more detailed account.

Perhaps the first realization that viruses infecting the same host plant reacted upon each other was the discovery of the so-called 'acquired immunity' against virus infection, or 'cross-protection' as it is more usually called. This is a phenomenon which is restricted to strains of the same viruses or at least to viruses having many properties in common.

The ability of one virus strain to inhibit the entrance of another strain into the same plant was first demonstrated by McKinney (1929), who showed that plants infected with a strain of tobacco mosaic virus which gave rise to a light-green mosaic underwent no change in symptoms after repeated inoculations with a strain of virus causing a yellow mosaic. In 1931 Thung carried out a similar experiment with a strain of tobacco mosaic virus causing a white mosaic

against the ordinary green mottling type. The same cross-protection phenomenon was also demonstrated by Salaman (1933) using different strains of potato virus X on *Datura stramonium*. It appeared at first from those experiments that a useful means of recognition of relationship between viruses was thus available. However, more work has not supported this hope, and the best that can be said for the method is that a relationship between two viruses can be presumed when there is cross-protection, but the absence of this phenomenon does not imply that the viruses are unrelated. Indeed, the range of reactions between related viruses in the same plant is very wide. Bennett (loc. cit.) has classified this range into (1) high degree of protection, (2) intermediate degree of protection, (3) low degree of protection. In the first category are mostly mosaic and ringspot-type viruses such as cucumber mosaic virus in cowpea and zinnia (Price, 1939; Fulton, 1950), and some strains of tobacco ringspot virus (Price, 1932; Tall, Price & Westmann, 1949). The second category is well represented by potato virus X which could also be included in the first category because of its variability in cross-protection. Thus Smith (1933) demonstrated the failure of a mottle strain to protect against a necrotic ringspot strain and Bawden & Sheffield (1944) found that potato plants infected with virus X were not completely protected against virus B. Similarly, Hutton (1948) tested a number of strains of potato virus X, some of which gave complete protection and some none at all, this depending in one case on the species of host plant. Tobacco mosaic virus strains also differ in the degree of protection afforded, and necrotic-type strains sometimes produce local lesions on leaves systemically infected with a mottling strain (Fulton, 1951).

The virus with the lowest degree of interference is that of curly-top of sugar beet, and there is little evidence of any tendency to acquire resistance by one strain of this virus against another (Giddings, 1950).

There is evidence in some cases that the degree of cross-protection between like viruses is correlated with their sero-

logical reactions. Matthews (1949) working with a number of strains of potato virus X found complete protection in the plant with those strains which gave the same serological reaction, but protection was not complete with those strains which differed serologically. On the other hand, this is not always the case; it has been shown that the viruses known as cucumber viruses 3 and 4 (cucumber green mottle mosaic and cucumber aucuba mosaic viruses) have similar serological, morphological and physical properties to tobacco mosaic virus (Bawden & Pirie, 1937; Knight & Stanley, 1941), but the presence of these viruses in the cotyledons of cucumber plants gives no protection against infection with tobacco mosaic virus (Fulton, 1951). Similarly tobacco veinal necrosis virus is serologically related to potato viruses Y and C. It does not protect tobacco, *Nicotiana glutinosa*, or potato plants from infection by them, and tobacco and *N. glutinosa* plants infected with either virus Y or C are still susceptible to it (Bawden & Kassanis, 1951).

Some interactions also occur between unrelated viruses acting together in the same plant, and these may take various forms. Suppression of, or antagonism towards, one of the two viruses has been described in one or two cases, whilst in others there may be increased severity of the disease or increased virus concentration.

Bawden & Kassanis (1945) have shown that the presence of the severe etch virus in tobacco plants prevents the multiplication of the two unrelated viruses of potato Y and henbane mosaic. Moreover, the severe etch virus is able to replace the two latter viruses in the plant. Bawden & Kassanis suggest that this may be due to an effect by the etch virus on cell metabolism resulting in the suppression of production of some material or enzyme system necessary for the increase of the potato Y and henbane mosaic viruses.

Another example of this kind of antagonism between unrelated viruses has been described by Ross (1950). He found that in the potato seedling U.S.D.A. 41956, which

is resistant to potato virus X, lesion formation by potato virus Y was partially inhibited if the inoculum contained potato virus X. Furthermore, the number of lesions produced by virus Y was progressively reduced as the concentration of virus X in the inoculum was increased.

According to McKinney (1940, 1941), the number of local lesions produced on the leaves of tobacco and *Nicotiana sylvestris* were reduced or delayed if the plants were already infected with cucumber mosaic virus. On the other hand, Garces-Orejuela & Pound (1957) state that in plants doubly infected with cucumber and tobacco mosaic viruses, the concentration of the latter was always higher in doubly than in singly infected plants, and more so after 1 or 2 weeks than after 3 or 4. Cucumber mosaic virus was more concentrated 4 days after inoculation in singly than in doubly infected plants, but after 2 weeks this was reversed, symptoms being more severe. In each of the combinations tested neither virus was able completely to inhibit increase of the other, and the enhanced symptoms were an additional effect.

A good example of increased severity of disease by a mixed virus infection is that known as 'double-virus streak' or 'glasshouse streak' of tomatoes. The two viruses concerned are that of tomato (= tobacco) mosaic and potato virus X. When occurring separately in tomato plants the diseases caused by these two viruses are not severe being mainly of the mottling type; when the two are acting together, however, much necrosis of the leaves and stem develops and frequently causes the death of the plant. Curiously enough the severity of the disease does not seem to vary, even if the component viruses are very mild strains.

A similar synergistic example is found in the potato disease known as crinkle produced by the two potato viruses A and X acting together (Murphy & McKay, 1932).

It has been shown by Ross (1950) and his co-workers that potato or tobacco plants doubly infected with potato virus X (PVX) and with potato virus Y (PVY) contained considerably more PVX than did comparable singly infected

plants. With leaves invaded while they were growing rapidly, i.e. during the acute stage of infection, ratios of 10 : 1 were obtained in several experiments in which the viruses were introduced together. This ratio may drop to 4 : 1 in leaves formed subsequent to systemic infection. There seems to be no comparable increase in concentration of the accompanying PVY.

Where there is a multiplicity of reactions there are likely to be many different mechanisms involved and there must be competition between two viruses operating in the same cell. Ross (1959) suggests that in the case of marked stimulation in the multiplication of a virus in a mixed infection this may be because the second virus either supplies specific substrates or prevents the action of by-products that are formed during the synthesis of the first virus and normally act to limit its multiplication.

REFERENCES

BAWDEN, F. C. and KASSANIS, B. (1945). 'The suppression of one plant virus by another.' *Ann. appl. Biol.* **32**, 52–57.

(1951). 'Serologically related strains of potato virus Y that are not mutually antagonistic in plants.' *Ann. appl. Biol.* **38**, 402–10.

BAWDEN, F. C. and PIRIE, N. W. (1937). 'The relationship between liquid crystalline preparations of cucumber viruses 3 and 4 and strains of tobacco mosaic virus.' *Brit. J. exp. Path.* **18**, 275–91.

BAWDEN, F. C. and SHEFFIELD, F. M. L. (1944). 'The relationship of some viruses causing necrotic diseases of the potato.' *Ann. appl. Biol.* **31**, 33–40.

BENNETT, C. W. (1927). 'Virus diseases of raspberry.' *Mich. Agric. Exp. Sta. Tech. Bull.* no. 80.

(1940). 'Relation of food translocation to movement of virus of tobacco mosaic.' *J. agric. Res.* **60**, 361–90.

(1953). 'Interactions between viruses and virus strains.' *Advances in Virus Research*, **1**, 40–67.

CALDWELL, J. (1930). 'The physiology of virus diseases in plants. I. The movement of mosaic in the tomato plant.' *Ann. appl. Biol.* **17**, 429–43.

(1934). 'The physiology of virus diseases in plants. VI. Some effects of mosaic on the metabolism of the tomato.' *Ann. appl. Biol.* **21**, 206–24.

CLINCH, P. (1932). 'Cytological studies of potato plants affected with certain virus diseases.' *Sci. Proc. Roy. Dublin Soc.* N.S. **20**, 143–72.

COOK, M. T. (1931). 'The effect of mosaic on cell structure and chloroplasts.' *J. Dept. Agric. Puerto Rico*, **15**, 177–81.

FULTON, R. W. (1950). 'Cross protection tests with cucumber viruses 3 and 4 and tobacco mosaic virus.' *Phytopathology*, **40**, 219–20.

(1951). 'Superinfection by strains of tobacco mosaic virus.' *Phytopathology*, **41**, 578–92.

GARCES-OREJUELA, C. and POUND, G. S. (1957). 'The multiplication of tobacco mosaic virus in the presence of cucumber mosaic virus or tobacco ringspot virus in tobacco.' *Phytopathology*, **47**, 232–9.

GIDDINGS, N. J. (1950). 'Some inter-relationships of virus strains in sugar beet curly top.' *Phytopathology*, **40**, 377–88.

HOUSTON, B. R., ESAU, K. and HEWITT, W. B. (1947). 'The mode of vector feeding and the tissues involved in the transmission of Pierce's disease virus in grape and alfalfa.' *Phytopathology*, **37**, 247–54.

HUTTON, E. M. (1948). 'The separation of strains from a virus X complex by passage through potato seedlings.' *Austral. J. sci. Res.* **B1**, 439–51.

KASSANIS, B., TINSLEY, T. W. and QUAK, F. (1958). 'The inoculation of tobacco callus tissue with tobacco mosaic virus.' *Ann. appl. Biol.* **46**, 11–19.

KNIGHT, C. A. and STANLEY, W. M. (1941). 'Aromatic amino acids in strains of tobacco mosaic virus and in the related cucumber viruses 3 and 4.' *J. biol. Chem.* **141**, 39–49.

KUNKEL, L. O. (1939). 'Movement of tobacco mosaic virus in tomato plants.' *Phytopathology*, **29**, 684–700.

MATTHEWS, R. E. F. (1949). 'Criteria of relationship between plant virus strains.' *Nature, Lond.* **163**, 175.

McKINNEY, H. H. (1929). 'Mosaic diseases in the Canary Islands, West Africa and Gibraltar.' *J. agric. Res.* **39**, 557–78.

(1940). 'The acquired-immunity test and its limitations for establishing relationship between virus mutants, and non-relationship between distinct viruses.' *Rep. Proc. 3rd Internat. Cong. Microbiol.* New York (1939), p. 316.

(1941). 'Virus-antagonism tests and their limitation for establishing relationship between mutants and non-relationship between distinct viruses.' *Amer. J. Bot.* **28**, 770–8.

MÜNCH, E. (1930). *Die Stopphewegenugen in der Pflanze*, 234 pp.

MURPHY, P. A. and McKAY, R. (1932). 'The compound nature of crinkle and its production by means of a mixture of viruses.' *Sci. Proc. Roy. Dublin Soc.* N.S. **20**, 227–47.

OWEN, P. C. (1955*a*). 'The respiration of tobacco leaves in the 20-hour period following inoculation with tobacco mosaic virus.' *Ann. appl. Biol.* **43**, 114–21.

(1955*b*). 'The respiration of tobacco leaves after systemic infection with tobacco mosaic virus.' *Ann. appl. Biol.* **43**, 265–72.

(1956). 'The effect of infection with tobacco mosaic virus on the respiration of tobacco leaves of varying ages in the period between inoculation and systemic infection.' *Ann. appl. Biol.* **44**, 227–32.

(1957). 'The effect of infection with tobacco etch virus on the rates of respiration and photosynthesis of tobacco leaves.' *Ann. appl. Biol.* **45**, 327–31.

PRICE, W. C. (1932). 'Acquired immunity to ringspot in *Nicotiana*.' *Contr. Boyce Thompson Inst.* **4**, 359–403.

PRICE, W. C. (1939). 'Cross protection tests with two strains of cucumber mosaic virus.' *Phytopathology*, **29**, 903–5.

ROSS, A. F. (1950). 'Unrelatedness of potato virus Y and cucumber mosaic virus.' *Phytopathology*, **40**, 445–52.

(1959). In 'Plant Pathology-Problems and Progress, 1908–1958.' *Phytopathology*, Jubilee Vol. Symposia.

SALAMAN, R. N. (1933). 'Protective inoculation against a plant virus.' *Nature, Lond.* **131**, 468.

SHEFFIELD, F. M. L. (1933). 'The development of assimilatory tissue in Solanaceous hosts infected with aucuba mosaic of tomato.' *Ann. appl. Biol.* **20**, 57–69.

SMITH, K. M. (1933). 'The present status of plant virus research.' *Biol. Rev.* **8**, 136–79.

(1935). 'Two strains of streak; a virus affecting the tomato plant.' *Parasitology*, **27**, 450–60.

(1946). 'The transmission of a plant virus complex by aphids.' *Parasitology*, **37**, 131–4.

TALL, M. G., PRICE, W. C. and WESTMAN, K. (1949). 'Differentiation of tobacco and tomato ringspot viruses by cross immunization and complement fixation.' *Phytopathology*, **39**, 288–9.

THUNG, T. H. (1931). 'Smetstof en plantencel bij enkele virusziekten van de Tabaksplant.' *Handelingen 6 de Nederle-Inde. Naturwetensch. Cong.* 450–63.

WHITEHEAD, T. (1934). 'The physiology of potato leaf-roll. I. On the respiration of healthy and leaf-roll infected potatoes.' *Ann. appl. Biol.* **21**, 48–77.

CHAPTER VI

Latent Virus Infections in Plants

DEFINITION

A good deal of confusion has arisen concerning the exact terms to be used to describe that phenomenon whereby an organism is infected with a virus but yet shows no apparent signs of infection. In order to clarify the situation a symposium was held in Wisconsin on 'Latency and Masking in Viral and Rickettsial Infections' (1957) and a symposium on similar lines was held in Stockholm on the occasion of the VIIth International Congress of Microbiology (1958).

The conclusions arrived at by the Wisconsin meeting are as follows:

Inapparent infection covers, at the host-parasite level, the whole field of infections which give no overt sign of their presence. Sub-clinical can be used as an alternative, particularly in human medicine.

Latent infections are inapparent infections which are chronic and in which a certain virus-host equilibrium is established. The adjective 'latent' is best reserved to qualify 'infection', the term 'latent virus' being avoided.

Occult virus is used to describe the cases where virus particles cannot be detected and in which the actual state of the virus cannot as yet be ascertained. It is preferred to 'masked', since this word has been used in a number of contradictory meanings.

Whenever it has been shown that viruses of animals or higher plants go through cycles as described for bacteriophage, the terms *provirus*, *vegetative virus* and *infective virus* are appropriate for the corresponding stages. Infective virus is the fully formed virus particle.

A *moderate* virus is one growing in a cell while still permitting its continued survival and multiplication; a *cytocidal* one kills the cell; *submoderate* covers intermediate cases. (Some viruses may be moderate in one cell-system, cytocidal in another, as, for example, potato virus X in different potato varieties.) However, as pointed out by Lwoff (1958), in order that an infection, whether apparent or not, should be recognized as viral, infectious particles have to be detected and identified as a virus.

In his contribution to the symposium on latency at the VIIth International Congress of Microbiology, Bennett (1959) prefers the term 'masking' and defines this as a condition in which a virus is actively present in a plant without causing obvious effects, regardless of the cause of this lack of obvious effects.

On the other hand, Bawden (1958) refers to the freedom of an infected plant from visible lesions as 'commensalism'. He considers this term more appropriate because it conveys the idea of existing together in harmony, and does not suggest, as do latency and masking, that the lack of virulence depends on some change in the state of the virus.

Smith (1952) suggests two types of latent virus infection in plants: in the first there is an initial reaction which soon disappears and the plant appears normal. Examples of this kind of infection are given by the tomato black ring virus (Smith, 1946) and viruses of the tobacco ringspot type. In the second category belong those viruses which never cause a disease in their original plant host and cannot apparently be made to do so. Examples of this group are the paracrinkle virus in King Edward potato (Salaman & Le Pelley, 1930), the latent infection in dodder, *Cuscuta* sp. (Bennett, 1944) and the latent infection in sugar-beets and mangolds (Smith, 1951).

CAUSES OF LATENT VIRUS INFECTIONS IN PLANTS

It is probable that the underlying causes of latency differ in the two types mentioned above. Where there is an initial

reaction which is followed by the disappearance of all symptoms the probable cause is low or reduced virus concentration as suggested by Bennett (1959), who considers that a drop in concentration may be associated with accumulation and rate of production of materials for virus synthesis. This type of latent infection can sometimes be stimulated once more to activity, for example, by the addition of another virus. In tomato plants which have completely recovered, so far as outward signs are concerned, from infection with the dodder latent virus, the concentration of the virus is very low. With the addition of tobacco mosaic or tobacco streak viruses to the recovered plants, however, symptoms of the dodder virus reappear and the concentration of this virus rises again to relatively high levels (Bennett, 1949). An interesting relationship exists between different types of orange and the tristeza virus. Both sweet and sour orange are highly resistant to injury when on their own roots, so that each species appears to have a high degree of tolerance of the concentration of virus it produces in its own tissues. However, experiments with aphid transfers suggest that virus concentration is much higher in the sweet than in the sour orange. When a graft is made of a sour orange top on a sweet orange rootstock, the tree remains symptom-free, but if the reverse procedure is carried out the tree is killed. When the virus concentration in the top is low and the root is tolerant as in sour orange on sweet, no damage is caused. However, if the virus concentration in the top is high and root tolerance is low as in the sweet orange or sour, death ensues (Bennett, 1959).

In the other type of latent infection such as the sugar-beet virus previously mentioned where there has been no initial disease, addition of a second virus has no stimulatory effect. The underlying causes for this type of latent infection are not known; it may be that by long association with a particular plant host a state of equilibrium or 'commensalism' has been reached.

A *moderate* virus is one growing in a cell while still permitting its continued survival and multiplication; a *cytocidal* one kills the cell; *submoderate* covers intermediate cases. (Some viruses may be moderate in one cell-system, cytocidal in another, as, for example, potato virus X in different potato varieties.) However, as pointed out by Lwoff (1958), in order that an infection, whether apparent or not, should be recognized as viral, infectious particles have to be detected and identified as a virus.

In his contribution to the symposium on latency at the VIIth International Congress of Microbiology, Bennett (1959) prefers the term 'masking' and defines this as a condition in which a virus is actively present in a plant without causing obvious effects, regardless of the cause of this lack of obvious effects.

On the other hand, Bawden (1958) refers to the freedom of an infected plant from visible lesions as 'commensalism'. He considers this term more appropriate because it conveys the idea of existing together in harmony, and does not suggest, as do latency and masking, that the lack of virulence depends on some change in the state of the virus.

Smith (1952) suggests two types of latent virus infection in plants: in the first there is an initial reaction which soon disappears and the plant appears normal. Examples of this kind of infection are given by the tomato black ring virus (Smith, 1946) and viruses of the tobacco ringspot type. In the second category belong those viruses which never cause a disease in their original plant host and cannot apparently be made to do so. Examples of this group are the paracrinkle virus in King Edward potato (Salaman & Le Pelley, 1930), the latent infection in dodder, *Cuscuta* sp. (Bennett, 1944) and the latent infection in sugar-beets and mangolds (Smith, 1951).

CAUSES OF LATENT VIRUS INFECTIONS IN PLANTS

It is probable that the underlying causes of latency differ in the two types mentioned above. Where there is an initial

reaction which is followed by the disappearance of all symptoms the probable cause is low or reduced virus concentration as suggested by Bennett (1959), who considers that a drop in concentration may be associated with accumulation and rate of production of materials for virus synthesis. This type of latent infection can sometimes be stimulated once more to activity, for example, by the addition of another virus. In tomato plants which have completely recovered, so far as outward signs are concerned, from infection with the dodder latent virus, the concentration of the virus is very low. With the addition of tobacco mosaic or tobacco streak viruses to the recovered plants, however, symptoms of the dodder virus reappear and the concentration of this virus rises again to relatively high levels (Bennett, 1949). An interesting relationship exists between different types of orange and the tristeza virus. Both sweet and sour orange are highly resistant to injury when on their own roots, so that each species appears to have a high degree of tolerance of the concentration of virus it produces in its own tissues. However, experiments with aphid transfers suggest that virus concentration is much higher in the sweet than in the sour orange. When a graft is made of a sour orange top on a sweet orange rootstock, the tree remains symptom-free, but if the reverse procedure is carried out the tree is killed. When the virus concentration in the top is low and the root is tolerant as in sour orange on sweet, no damage is caused. However, if the virus concentration in the top is high and root tolerance is low as in the sweet orange or sour, death ensues (Bennett, 1959).

In the other type of latent infection such as the sugar-beet virus previously mentioned where there has been no initial disease, addition of a second virus has no stimulatory effect. The underlying causes for this type of latent infection are not known; it may be that by long association with a particular plant host a state of equilibrium or 'commensalism' has been reached.

SOME EXAMPLES OF LATENT VIRUS INFECTIONS

One of the earliest examples was described by Johnson (1925) and was called the 'healthy potato virus'. This apparent contradiction in terms was used to designate what is now universally known as 'potato virus X'; this virus was carried without visible symptoms by many of the American potato varieties. Latent infections of this type depend as much on the host as on the virus, because in some potato varieties the X virus may cause severe disease.

As would perhaps be expected, chronic latent infection is commonest in plants which are vegetatively propagated. No doubt the continual propagation of the virus in the same host helps to bring about a state of equilibrium between virus and host plant.

We have seen in the case of potato virus X that 'virulence' or latency are as much a function of the host plant as of the virus, since the same virus may be latent in one potato variety and virulent in another. This kind of reaction occurs with other plant viruses; a certain variety of dahlia, Bishop of Llandaff, for example, will carry the virus of cucumber mosaic without symptoms, whilst another dahlia variety will give a mosaic mottle with the same virus. Certain varieties of the hop plant, notably Fuggles, have a latent infection with the hop mosaic virus, whereas on the Goldings hop the same virus is virulent. Latent infections are common also in raspberry and strawberry plants.

Good examples of latent infection are found among the soil-transmitted viruses, those of the tobacco necrosis type occurring without symptoms in the roots of many plant species.

Similarly the soil-transmitted ringspot viruses cause latent infections in a number of common weeds (Cadman, 1956).

ECONOMIC SIGNIFICANCE OF LATENT VIRUS INFECTIONS

It is fairly obvious that, if a virus-diseased plant is in the vicinity of a susceptible crop and if, as is probable, there exist one or more methods by which the virus can spread in the field, the susceptible crop is likely to become virus-diseased. In such a case it is possible to take some remedial measures like the elimination of the source of virus or the control of a potential vector. The situation becomes more serious when the source of virus is a latent infection, and here, of course, the first step is to locate the source, and this is not always easy. For example, it was some years before the serious disease of raspberry plants in Scotland, known as 'yellow dwarf', was found to be due to a soil-transmitted virus of the 'ringspot' type carried without symptoms by a number of common weeds (Cadman, 1959).

It is among fruit trees, and more especially stone fruits, that the latent infection is of great economic importance. So often the root-stock used in grafting harbours one or more latent infections and large numbers of young trees become thus infected without the grower's knowledge.

Among stone-fruit trees, the peach X virus, the ringspot and ring mottle viruses in cherries and the bark-split virus in plums may be mentioned. The rubbery wood virus is latent in many apple varieties and sooty ringspot virus in pears. Two other viruses latent in pears have recently been discovered; these are the yellow blotch and bark necrosis viruses (Posnette & Cropley, 1958).

One of the most recent cases of devastating loss due to a virus infection which was latent in a fruit-tree stock but caused a serious disease in the grafted top is the tristeza disease of citrus trees already referred to. For example, in 1946 the virus was responsible, in the principal citrus-producing State of São Paulo in Brazil, for the loss of over 4 million trees. Up to 1937 the disease was not known in Brazil, but it was then recognized as identical with a 'root rot' responsible for very heavy damage in the northern

Argentina where it decimated the groves of sweet orange grafted on sour orange stocks. Originally known as a 'root rot' before its virus nature was recognized, the tristeza disease was first observed in South Africa about 1910, in Java in 1928, in Argentina about 1931 and Brazil in 1937. It is also widespread in Israel and the U.S.A. Now, however, it is possible to take some control measures since it is known that the virus is latent in the sour orange root-stock and since the insect vectors, *Aphis citricidus* Kirk and *A. gossypii* Glover, have been identified.

REFERENCES

BAWDEN, F C. (1958). 'Gradations and transitions between pathogenicity and commensalism in infections with plant viruses.' *Sympos. Latency and Masking in Viral and Rickettsial Infect.* pp. 80–87. Minneapolis, U.S.A.: Burgess Publishing Co.

BENNETT, C. W. (1944). 'Latent virus of dodder and its effect on sugar beet and other plants.' *Phytopathology*, **34**, 77–91.

(1949). 'Recovery of plants from dodder latent mosaic.' *Phytopathology*, **39**, 637–46.

(1959). 'Masked plant viruses.' *Rep. Proc. VIIth Internat. Cong. Microbiol.* 1958, Stockholm, pp. 218–23.

CADMAN, C. H. (1956). 'Studies on the etiology and mode of spread of raspberry leaf curl disease.' *J. hort. Sci.* **31**, 111–18.

(1959). Personal communication.

JOHNSON, J. (1925). 'The transmission of viruses from apparently healthy potatoes.' *Wisc. Agric. Exp. Sta. Res. Bull.* no. 63.

LWOFF, A. (1958). 'Remarks on the terminology of viruses and of viral infections.' *Sympos. Latency and Masking in Viral and Rickettsial Infect.* pp. 185–9. Minneapolis, U.S.A.: Burgess Publishing Co.

POSNETTE, A. F. and CROPLEY, R. (1958). 'Quince indicators for pear viruses.' *J. hort. Sci.* **33**, 289–91.

'Recent Progress in Microbiology. Sympos. IV. Latent and Masked virus infections.' *Rep. Proc. VIIth Internat. Cong. Microbiol.* 1958, Stockholm.

SALAMAN, R. N. and LE PELLEY, R. H. (1930). 'Paracrinkle; a potato disease of the virus group.' *Proc. roy. Soc. Lond. B*, **106**, 140–75.

SMITH, K. M. (1946). 'Tomato black ring; a new virus disease.' *Parasitology*, **37**, 126–30.

(1951). 'A latent virus in sugar beets and mangolds.' *Nature, Lond.* **167**, 1061.

(1952). 'Latency in viruses and the production of new virus diseases.' *Biol. Rev.* **27**, 347–57.

Symposium on Latency and Masking in Viral and Rickettsial Infections. 1958. Minneapolis, U.S.A.: Burgess Publishing Co.

CHAPTER VII

Electron Microscopy of Plant Viruses

SIZE AND SHAPE OF VIRUS PARTICLES

The old definition which spoke of the 'filterable ultramicroscopic viruses' is now, of course, long out of date. Filter membranes can be made, with the pore size adjusted to hold back or allow to pass any virus (Elford, 1931), and the modern electron microscope can easily resolve the smallest virus known.

There are many methods other than by means of the electron microscope for measuring the particle size of viruses, and for information on these the reader is referred to a paper by Markham, Smith & Lea (1942) or to Bawden (1950).

It was as long ago as 1932 that the first suggestion was made regarding the shape of a plant-virus particle. Sols containing rod-shaped particles are doubly refractive when the particles are orientated by streaming and the direction of observation is perpendicular to the direction of flow. Takahashi & Rawlins (1932) observed the flow of tobacco mosaic sap through crossed nicols and found that the sap showed double refraction or anisotropy of flow. They were thus the first to suggest that tobacco mosaic virus had rod-shaped particles, an observation which has since been amply confirmed by the electron microscope (Fig. 7). There is of course a certain amount of preliminary preparation necessary of the infected plant sap to separate the virus from host-cell materials before it is possible to observe it on the electron microscope. The extent of this preparation depends a good deal on the stability of the virus and its concentra-

tion in the plant. A short account of the preliminary treatments of virus-containing sap which are necessary for electron microscopy is given in Part II.

As might perhaps be expected, the sizes and shapes of the different viruses vary to a high degree. Some have rod-shaped particles as in tobacco mosaic virus or more string-like in appearance as with potato virus X. Others such as the viruses of tomato bushy stunt or turnip yellow mosaic are near-spherical, whilst one or two others may be somewhat irregular in shape.

Tobacco Mosaic Virus

This, the most studied of all plant viruses, was the first to be discovered, the first to be isolated and the first to be observed on the electron microscope.

Owing to the rather drastic methods used in the purification of tobacco mosaic virus the rods tend to aggregate side by side or end to end and also to break up into shorter lengths. In consequence, there has been some controversy as to the exact size of the particle of tobacco mosaic virus (Bawden & Pirie, 1945). In 1946 Oster & Stanley examined the virus directly from the hair cells of tobacco mosaic plants without any chemical treatment and found that 68 per cent. of the particles measured 280 mμ in length by 15 mμ in width. They concluded that these rods represent the minimal infective unit and occur as such within the cells of plants infected with this virus.

An immense amount of work has been carried out upon the structure of the particle of tobacco mosaic virus, much of it by means of X-ray diffraction studies, and only a brief mention of this work is possible here.

The X-ray studies by Franklin, Klug & Holmes (1957) and others have shown the virus particle to consist of a number of protein sub-units set in a helical array with 49 sub-units to one turn of the helix and 2,130 sub-units in one rod. The ribonucleic acid thread intertwines more or less centrally between the protein sub-units.

The results obtained by electron microscopy are rather

disappointing since micrographs reveal no sign of a helix but show only a straight rod exhibiting no regularity of detail in its surface structure (Fig. 8). It is possible, however, by means of staining with phosphotungstic acid to reveal the ribonucleic acid. Hart (1955) removed part of the protein coat of the virus rod by treatment with sodium dodecyl sulphate; this revealed a core of material axially localized where it joined the remaining portion of the intact rod. This core seems undoubtedly to be the protruding ribonucleic acid (RNA) since treatment with the enzyme RNase removed it, but treatment with DNase or trypsin had no effect.

Potato Virus X

This virus is also rod-shaped but appears to be much less rigid than the virus of tobacco mosaic and intertwines in a manner rather like pieces of string. High-resolution micrographs of single rods of potato virus X show it to be superficially similar to TMV, but nothing seems to be known of its internal structure (Fig. 9).

Other Rod-shaped Viruses

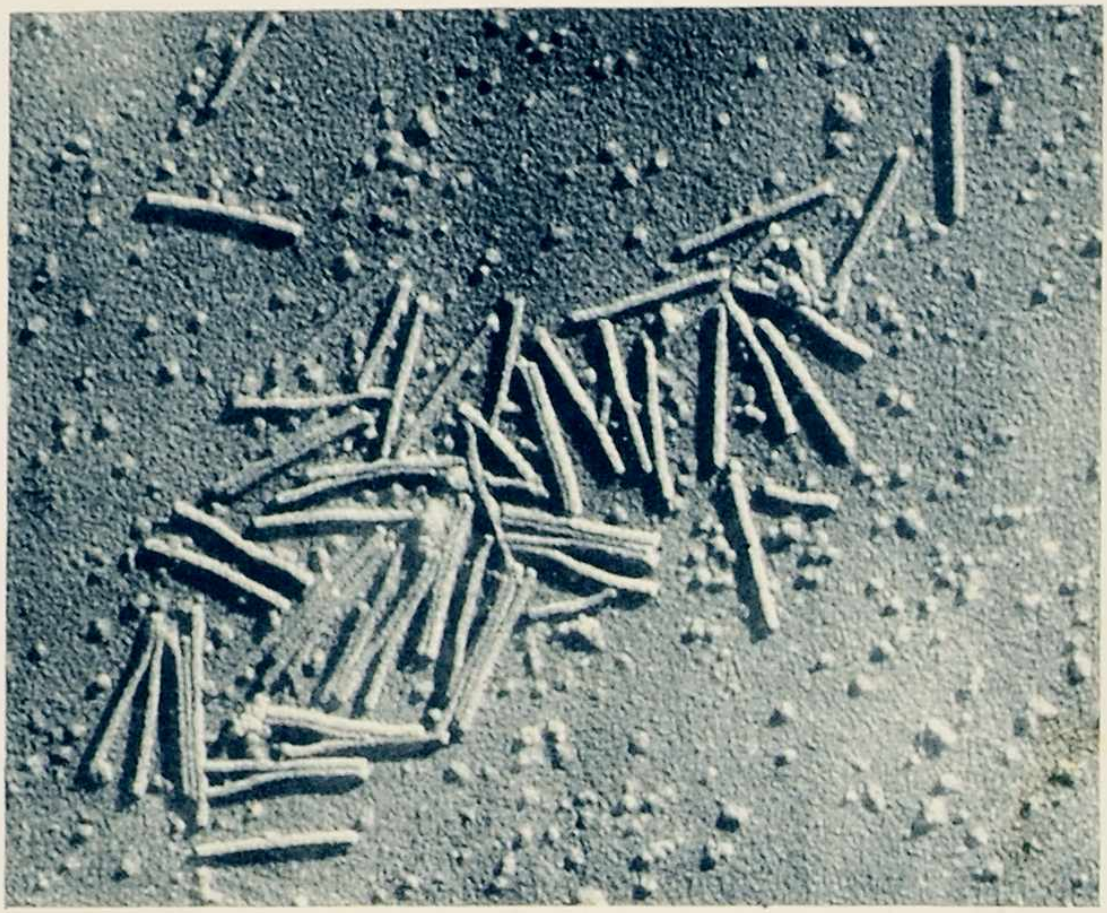
Quite a number of plant viruses have now been shown to be filiform; the cabbage black ringspot virus appears to consist of long flexuous rods (Larson *et al.*, 1950), which, according to Bode & Brandes (1958), have a length of 754 mμ and a diameter of 12–13 mμ. The Wisconsin pea streak virus consists of curved rods about 700 mμ in length (Stahman & Kaesberg, 1955). The virus of false stripe disease of barley has particles measuring 30 × 130 mμ (Gold, Suneson, Houston & Oswald, 1954). Very similar measurements for this virus were obtained by Kassanis & Slykhuis (1958), who describe it as consisting of stiff particles about 150 mμ in length by 30 mμ in diameter.

According to Mundry (1958), the beet yellows virus is of the filiform type, but no exact measurements are given.

The foregoing are mostly long flexible rods, but some viruses have quite short, rather thick particles as with a

Fig. 5 (above). Mottling and ring-like formations on leaf of delphinium, caused by infection with cucumber mosaic virus

Fig. 6 (below). Symptoms of yellow bean mosaic on French bean, Phaseolus vulgaris



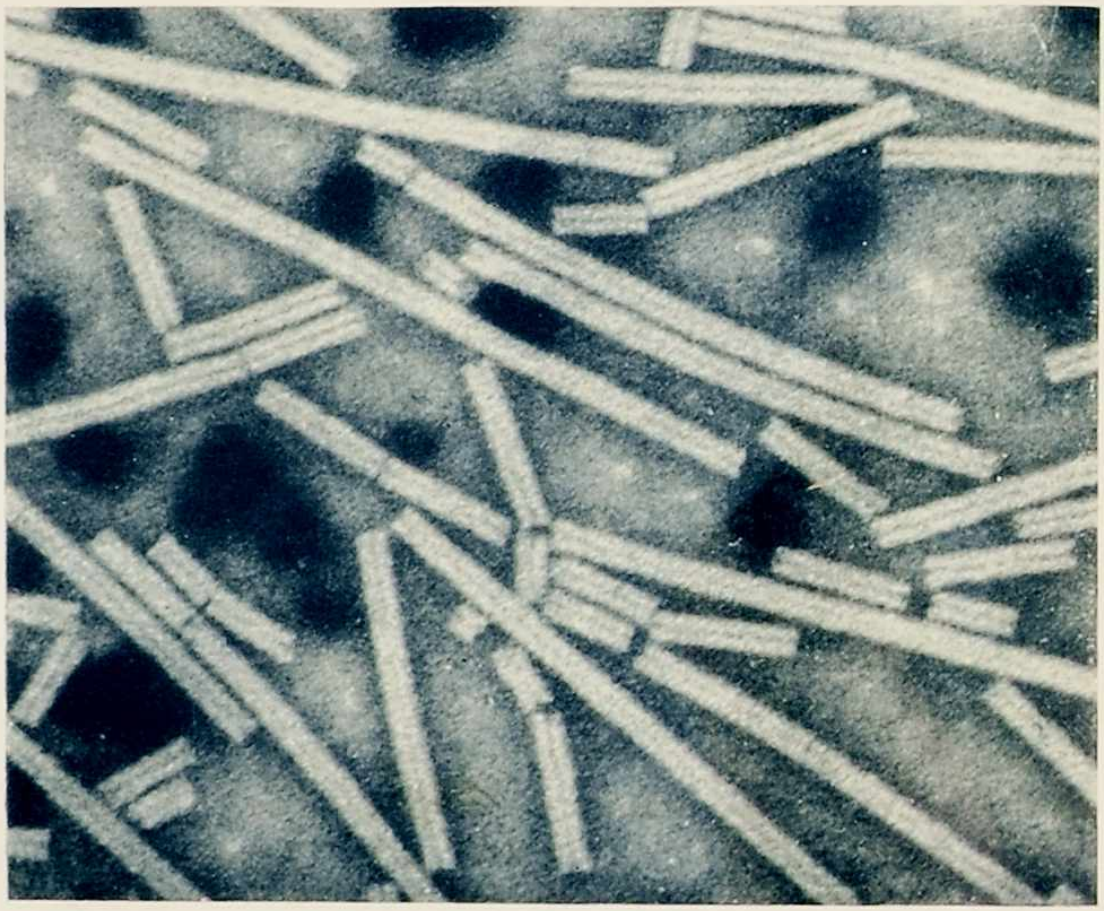


Fig. 7 (above). Tobacco mosaic virus particles, shadowed with palladium-gold. × 40,000. Fig. 8 (below). Tobacco mosaic virus particles stained with phosphotungstic acid; note the hollow centre picked out by the stain. × 133,000

virus causing a ringspot disease in New Zealand spinach and isolated originally from a cultivated species of *Ranunculus*. Similarly, the virus particles of alfalfa mosaic are short rods measuring 20 × 55 mμ (Bancroft & Kaesberg, 1958).

Spherical and Near-spherical Particles

Many plant virus particles have this shape, notably those of turnip yellow mosaic, tomato bushy stunt, tobacco ringspot, tobacco necrosis and others.

Whilst examining frozen-dried particles of squash mosaic and turnip yellow mosaic viruses, Stahman & Kaesberg (1955) noticed that they seemed to have a hexagonal contour. Kaesberg (1956) investigated this matter further and examined on the electron microscope lightly shadowed, frozen-dried preparations of purified turnip yellow mosaic, squash mosaic, wild cucumber mosaic (*Echinocystis lobata*) and brome grass mosaic viruses. All these viruses almost invariably suggested a polygonal contour under the electron microscope. Hexagonal contours were frequently seen in the squash, wild cucumber and brome grass mosaic viruses and occasionally in turnip yellow mosaic virus. The evidence from this material, combined with heavy shadowing with uranium, suggests that these four viruses may have approximately the shape of symmetrical icosahedra. From the particle contours this is best shown by the brome mosaic virus and least well by the turnip yellow mosaic virus (Kaesberg, 1956). On the other hand, so far as the last-named virus is concerned, Cosentino *et al.* (1956) considered the particles to be nearly spherical in shape with a diameter of 26 mμ.

Steere (1957) has developed a technique by which preshadowed replicas can be obtained from plant virus crystals which have been cut and then etched by sublimation of the ice from their surfaces. Electron micrographs of specimens prepared by this low-temperature replica procedure show that in a crystal of tobacco ringspot virus in 0·01M phosphate buffer the individual virus particles show a hexagonal

outline and hexagonal packing within the crystal. In a similar preparation of purified squash mosaic virus there is hexagonal packing, but the individual particles are not distinctly hexagonal as are those of the tobacco ringspot virus. The particles of turnip yellow mosaic virus also exhibit hexagonal packing, but each particle seems to have on it a number of uniform knobs.

Black (1955) has obtained pictures of the wound tumour virus from both the infected plant and the insect vector; it measures about 75 mμ across, from either source. Tomato spotted wilt virus seems to be slightly larger, measuring about 85 mμ in diameter; both these viruses show some tendency towards a polyhedral shape. The potato yellow dwarf viruses seem to be the largest yet described and the shape varies in different preparations. There seems to be no estimate of the approximate size of these virus particles.

It has been shown recently that viruses other than those affecting plants are also icosahedra, particularly the insect viruses (Williams & Smith, 1958; Hills & Smith, 1959). In addition, the shape of the animal viruses known as adenoviruses is apparently polyhedral (Valentine & Hopper, 1957).

THE PLANT VIRUS IN THE CELL

As it is impossible to examine living cells under the electron microscope, the next best thing is to examine fixed material of virus-infected cells. This has been made feasible by the development of a technique for cutting extremely thin sections by means of an ultra-microtome.

It is rather disappointing that more work on these lines has not been carried out; in consequence, our information on the behaviour of plant viruses in the cell is meagre compared with that obtained in comparable studies on the animal viruses. Most of the thin section work on virus-infected plant cells has been confined to tobacco mosaic virus. This is understandable, perhaps, because the extremely small size of many plant viruses makes it very

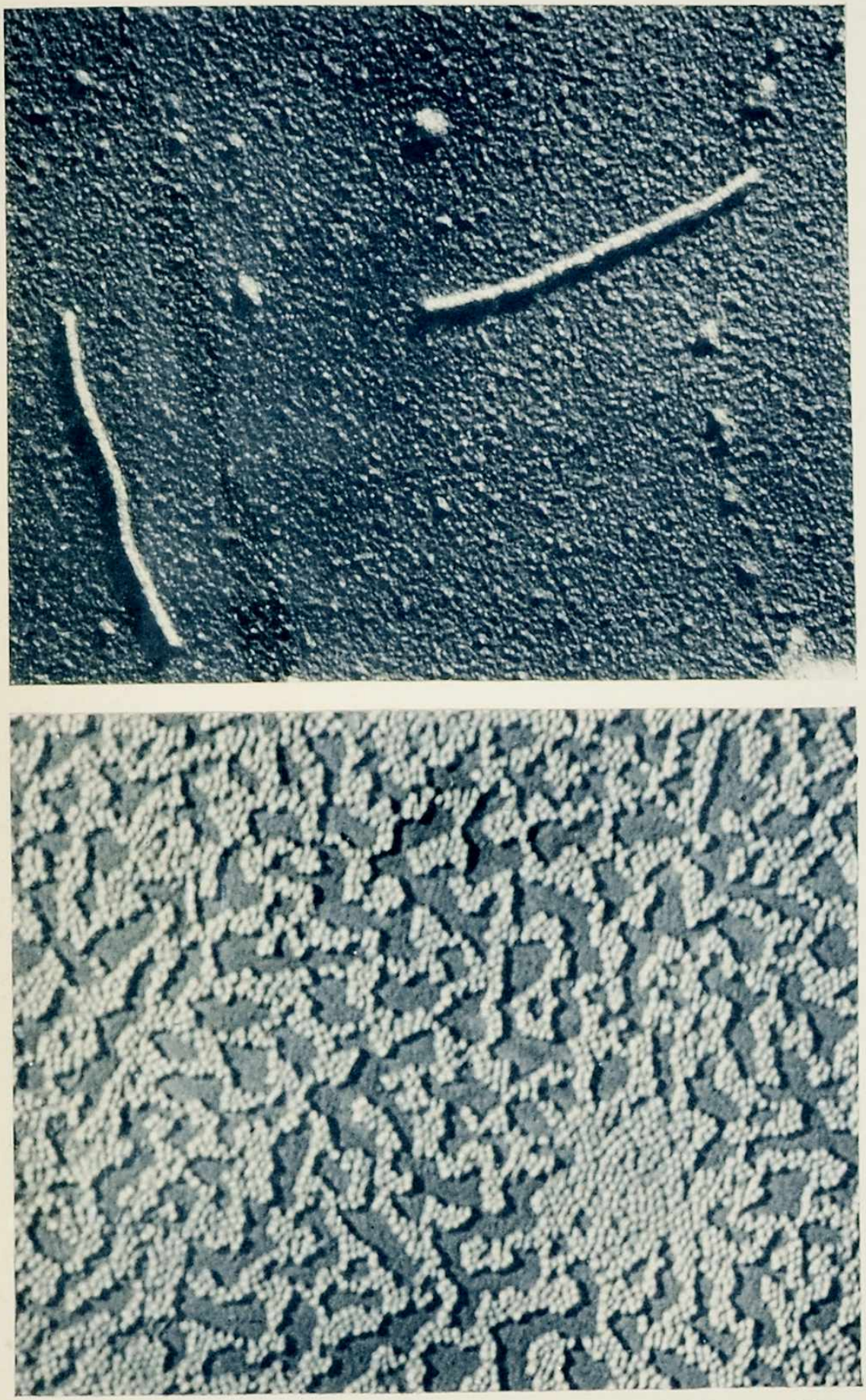
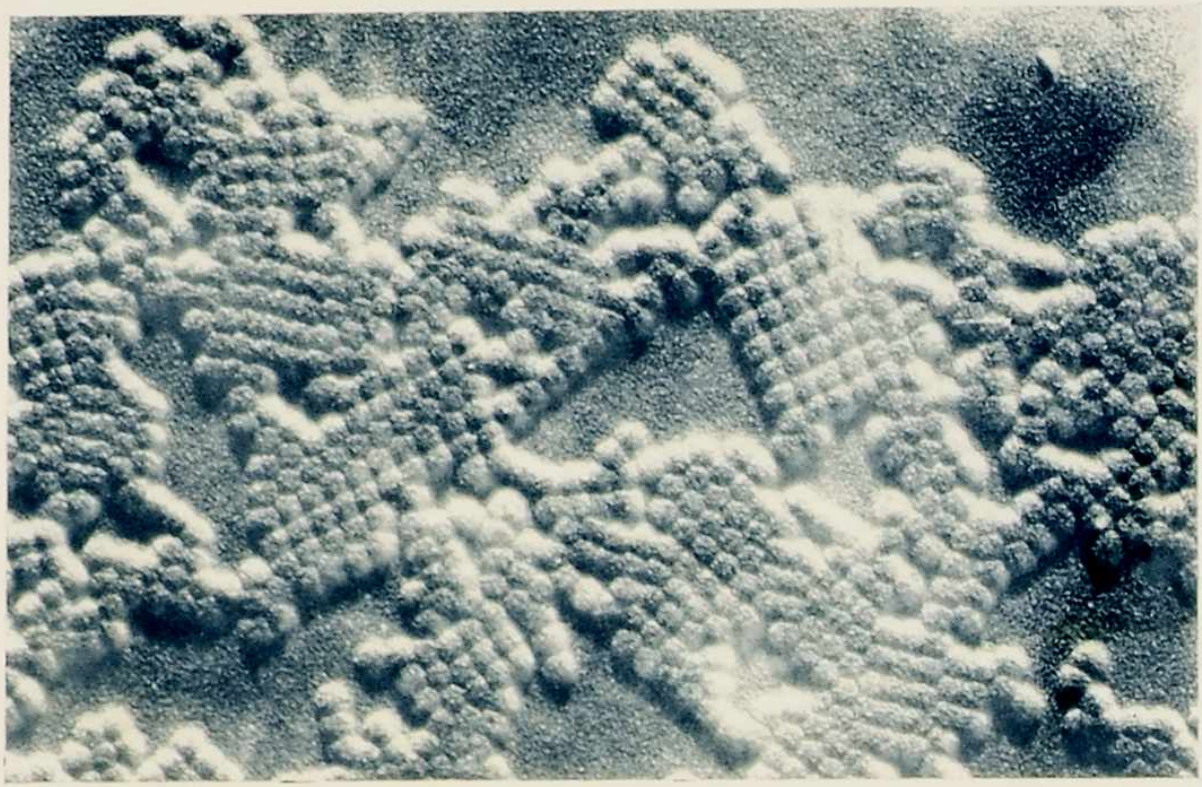


Fig. 9 (above). Potato virus X, shadowed with palladium-gold, the particles are less rigid than those of tobacco mosaic virus. × 80,000. Fig. 10 (below). Tomato bushy stunt virus, shadowed with palladium-gold. × 27,000. (Micrograph by S. Vernon-Smith)



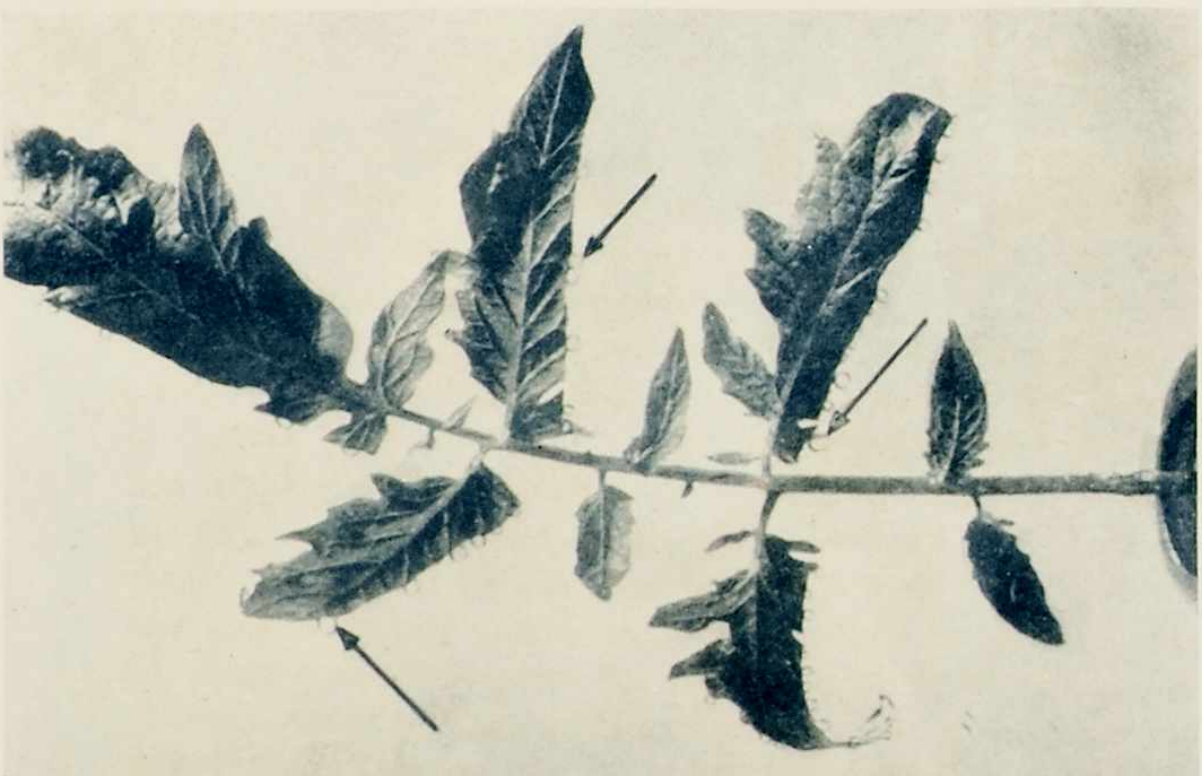


Fig. 11 (above). Turnip crinkle virus, carbon replica. × 80,000

Fig. 12 (below). Tomato leaf under water pressure, showing droplets used for preparation of electron microscope screens; leaflets cut for collection of vascular contents. (After J. Johnson)

difficult to differentiate them from normal cell constituents. Occasionally when the virus is in high concentration in the cell a micro-crystal may form and identification then becomes possible.

On the other hand, the characteristic shape of tobacco mosaic and other rod-shaped viruses renders them more easily visible, and Nixon (1956) has estimated the number of tobacco mosaic virus particles in a single hair cell by means of a complete longitudinal section.

There has been some controversy as to whether there is a close association between the tobacco mosaic virus particles and the chloroplasts, and suggestions have been made that the virus actually multiplies inside the chloroplasts. Skotland *et al.* (1955) thought that there might be an association of the chloroplast components and virus formation because cell sections from infected tissues showed evidence of frequent chloroplast disintegration and the release of the grana and other chloroplast contents into the cytoplasm. Virus, visible as rod-shaped particles, was observed most frequently in regions containing the grana.

Boardman & Zaitlin (1958) offer some evidence of the association of tobacco mosaic virus with plastids by methods other than the electron microscope. Their findings show the virus from the chloroplast fraction to be metabolically distinct from the virus as isolated from the remainder of the leaf homogenate, and they are consistent with the hypothesis that tobacco mosaic virus protein is synthesized within the chloroplast, or that the protein and nucleic acid portions of the virus are assembled within the chloroplast. On the other hand, Matsui (1958) has never observed virus rods in the chloroplasts and considers that the intimate association of virus rods with them could be interpreted as secondarily established. The writer (Smith, 1953) has not observed the small viruses of turnip yellow mosaic or tomato bushy stunt within the chloroplasts.

There is a latent period after the tobacco mosaic virus first enters a susceptible cell when it is thought that the

particle is denuded of its protein coat, leaving the free ribonucleic acid.

Sukhov and Kapitza (1957), quoted by Grabar (1958), have studied the initial stages of development of tobacco mosaic virus in the cell. During the latent period of infection, formation of fibrillar structures was observed. The presence of fibrillae of various lengths seems to suggest that they are growing by increase in length. The formation of spirals from these particles explains the increase of the diameter of the mature rods and the modification of their antigenic properties.

Probably with the development of new staining techniques for electron microscopy it will soon be possible to make more progress in this particular field.

ELECTRON MICROSCOPY OF PLANT VIRUS CRYSTALS

Very beautiful pictures of the surface of plant virus crystals can be obtained by the use of replicas. This method has been developed by Wyckoff (1949), Hall (1953) and others. Put very briefly, the technique consists in covering the object with a dilute solution of collodion or formvar and allowing it to evaporate to form a film. The object can then be dissolved in a liquid so as to leave the film floating on the surface; the film can then be picked up on the microscope grid. For plant viruses a carbon film evaporated onto the object gives a better result than collodion. In Fig. 11 is a replica of a plant virus crystal; it shows very clearly the arrangement of the virus particles in the crystal lattice. (See also Steere, 1957.)

REFERENCES

BANCROFT, J. B. and KAESBERG, P. (1958). 'Size and shape of alfalfa mosaic virus.' *Nature, Lond.* **181,** 720–1.

BAWDEN, F. C. (1950). *Plant Viruses and Virus Diseases.* Waltham, Mass.: Chronica Botanica Co.

BAWDEN, F. C. and PIRIE, N. W. (1945). 'Further studies on the purification and properties of a virus causing tobacco necrosis.' *Brit. J. exp. Path.* **26**, 277–85.

BLACK, L. M. (1955). 'Concepts and problems concerning purification of labile insect-transmitted plant viruses.' *Phytopathology*, **45**, 208–16.

BOARDMAN, N. K. and ZAITLIN, M. (1958). 'The association of tobacco mosaic virus with plastids. II. Studies on the biological significance of virus as isolated from a chloroplast fraction.' *Virology*, **6**, 758–68.

BODE, O. and BRANDES, J. (1958). 'Elektronenmikroskopische Untersuchung des Kohlrübenmosaik-Virus.' *Phytopath. Z.* **34**, 103–6.

COSENTINO, V., PAIGEN, K. and STEERE, R. L. (1956). 'Electron microscopy of turnip yellow mosaic virus and the associated abnormal protein.' *Virology*, **2**, 139–48.

ELFORD, W. J. (1931). 'A new series of graded collodion membranes suitable for general bacteriological use, especially in filterable virus studies.' *J. path. Bact.* **34**, 505.

FRANKLIN, ROSALIND, KLUG, A. and HOLMES, K. C. (1957). 'X-ray diffraction studies of the structure and morphology of the tobacco mosaic virus.' *The Nature of Viruses, Ciba Found. Sympos.* 39–52.

GOLD, A. H., SUNESON, C. A., HOUSTON, B. R. and OSWALD, J. W. (1954). 'Electron microscopy and seed and pollen transmission of rod-shaped particles associated with the false stripe virus disease of barley.' *Phytopathology*, **44**, 115–17.

GRABAR, P. (1958). *Ann. Rev. Microbiol.* **12**, 383–414.

HALL, C. E. (1953). *Introduction to Electron Microscopy*. New York: McGraw-Hill Publishing Co.

HART, R. G. (1955). 'Infectivity measurements of partially degraded tobacco mosaic virus.' *Virology*, **1**, 402–7.

HILLS, G. J. and SMITH, K. M. (1959). 'Electron microscopy of the insect cytoplasmic viruses.' *J. Insect. Path.* **1**, 121–8.

KAESBERG, P. (1956). 'Structure of small "spherical" viruses.' *Science*, **124**, 626–8.

KASSANIS, B. and SLYKHUIS, J. T. (1958). *Rep. Rothamsted Exp. Sta. 1957.*

LARSON, R. H., MATTHEWS, R. E F. and WALKER, J. C. (1950). 'Relationships between certain viruses affecting the genus *Brassica*.' *Phytopathology*, **40**, 955–62.

MARKHAM, R., SMITH, K. M. and LEA, D. E. (1942). 'The sizes of viruses and the methods employed in their estimation.' *Parasitology*, **34**, 315–52.

MATSUI, C. (1958). 'Pathological cytology of the tobacco leaf infected with tobacco mosaic virus.' *J. biophys. & biochem. Cytol.* **4**, 831.

MUNDRY, K. W. (1958). 'Über die Korrelation Zwischen Partikellänge und Infektiösität beim Vergilbungsvirus der Rüben.' *Z. Naturf.* **13bI**, 19–27.

NIXON, H. L. (1956). 'An estimate of the number of tobacco mosaic virus particles in a single hair cell.' *Virology*, **2**, 126–8.

OSTER, G. and STANLEY, W. M. (1946). 'An electron microscope study of the contents of hair cells from leaves diseased with tobacco mosaic virus.' *Brit. J. exp. Path.* **27**, 261–5.

SKOTLAND, C. B., HAGEDORN, D. J. and STAHMANN, M. A. (1955) 'Electron microscopy of tobacco mosaic virus *in situ*.' *Phytopathology*, **45**, 603–7.

SMITH, K. M. (1953). 'A note on the observation of viruses in the cells of infected plants.' *Biochim. Biophys. Acta*, **10**, 210–14.

STAHMAN, M. A. and KAESBERG, P. (1955). 'Concepts and problems concerning the electron microscopy of plant viruses.' *Phytopathology*, **45**, 187–95.

STEERE, R. L. (1957). 'Electron microscopy of structural detail in frozen biological specimens.' *J. biophys. & biochem. Cytol.* **3**, 45–60.

SUKHOV, K. S. and KAPITZA, O. S. (1957). *Doklady Akad, Nauk. S.S.S.R.* **113**, 1366–8.

TAKAHASHI, W. N. and RAWLINS, T. E. (1932). 'A method

for determining the shape of colloidal particles; application in the study of tobacco mosaic virus.' *Proc. Soc. exper. Biol. & Med.* **30,** 155–7.

VALENTINE, R. C. and HOPPER, P. K. (1957). 'Polyhedral shape of adenovirus particles as shown by electron microscopy.' *Nature, Lond.* **180,** 928.

WILLIAMS, R. C. and SMITH, K. M. (1958). 'The polyhedral form of the *Tipula* iridescent virus.' *Biochim. Biophys. Acta,* **28,** 464–9.

WYCKOFF, R. W. G. (1949). *Electron Microscopy.* New York: Interscience Publishing Co. Inc.

PART II

PRACTICAL METHODS IN PLANT VIROLOGY

CHAPTER VIII

Mechanical Methods of Inoculation

It may be as well to point out at the beginning that many viruses are not at present transmissible by mechanical methods of inoculation but are dependent upon specific insect vectors for their spread. The methods to be described here do not, therefore, apply to these viruses.

The word inoculation is used here in the restricted sense of the application of virus-bearing fluids to the tissues of susceptible plants. In the early days of plant virus study the standard method of inoculating plants with viruses was to place a few drops of crude extracted sap from the virus-infected plant upon the leaves of the plant to be infected and to scratch through the drop with a needle mounted in a handle. It was then realized that the virus could only enter through a wound, however slight – a broken trichome would suffice – and therefore the more points of entry for the virus the better. Furthermore, it has been calculated that a very large number of virus particles must be applied to a leaf surface before a single virus lesion is formed (Chester, 1935; Bawden, 1950). Steere (1955) considers that only one particle out of 50,000 produces an infection. Nevertheless, it has been suggested by Kunkel (1934) and by Lauffer & Price (1945) that each lesion of tobacco mosaic virus is caused by a single virus particle. It is clear therefore that, at the best, mechanical inoculation is an indifferent mode of virus transmission. In efforts to improve it a great many modifications have been evolved and are dealt with in this section.

A great improvement in the inoculation technique was made by Samuel (1931), who showed that a gentle rubbing,

almost a wiping, of the leaf to be inoculated gave a much higher infection percentage then the old needle method. A glass spatula with a ground-glass face dipped into the inoculum was recommended, the leaf to be inoculated being supported on a filter paper held in the hand. In practice, however, the tip of the forefinger serves the purpose of a spatula equally well.

A further advance was made when it was found that an abrasive, dusted lightly over the leaves before inoculation or added to the inoculum, greatly increased the points of entry of the virus (Rawlins & Tompkins, 1936). A fine grade of carborundum powder or diatomaceous earth such as celite are suitable for this purpose. It is important, however, to dust the leaves lightly and to use the minimum of pressure whilst rubbing the leaves. Certain species of plants, notably French beans (*Phaseolus vulgaris*) and cowpea (*Vigna sinensis*), are particularly liable to be damaged by the inoculation process. In 1929 Holmes demonstrated that if the inoculated leaves were washed with water immediately after inoculation, under a tap or by means of a 'squash' bottle, the number of local lesions or points of entry of the virus was greater than if the leaves were left unwashed after inoculation.

If unduly prolonged, however, the washing may result in a decrease in infection. A one-minute rinse may cause as much as 75 per cent. decrease in infection with tobacco mosaic virus (Dale, 1956). Yarwood (1955*a*) considers that a *short* washing of inoculated leaves immediately after inoculation increases infection, but reduces it if prolonged for more than 20 seconds.

To summarize, the routine inoculation of a fairly infectious sap-transmissible virus would be carried out as follows: dust the leaves to be inoculated *lightly* with celite or fine carborundum powder; dip the forefinger or a piece of muslin into the inoculum and rub the leaves *gently*, then wash the excess inoculum off the leaf surfaces.

Many modifications and special methods have been introduced for dealing with unstable viruses or those diffi-

cult to transmit mechanically. In addition, it has been shown that special treatment of plants before and after inoculation may have considerable influence on the reaction of the plants to infection. All these are described later in this section.

PREPARATION OF VIRUS INOCULUM

The routine method of preparing the inoculum consists in grinding the virus-infected leaves with a pestle and mortar; if a large quantity of inoculum is required an ordinary mincing machine may be used. The macerated leaf material is then placed in a piece of cheesecloth and the sap extracted by pressure with the fingers or the pestle. If a large amount of material is involved a screw press may be used. Yarwood (1957) describes a method applicable to viruses such as tobacco mosaic virus which occur in high concentration in the infected plant. He adds about one square centimetre of leaf tissue and about three drops of water to an ordinary mortar, grinds them thoroughly and dilutes them with additional water or phosphate solution as desired. This gives a suspension in which particulate matter is barely detectable with the unaided eye.

When only a very small quantity of inoculum is available, other methods can be used. Holmes (1952) suggests cutting out with a cork borer a small disc of leaf tissue, containing a single virus lesion if necessary, and grinding the disc between two pot labels. The macerated tissue can then be transferred directly to the healthy leaf by gently rubbing with the contaminated face of the label. Alternatively the disc can be transferred to a microscope slide with a ground-glass surface and macerated with a spatula with a similar surface.

Takahashi (1951) has designed a special type of pestle and mortar made of glass for grinding small quantities of material. This consists of a tapered, self-fitting ground-glass homogenizer of simple design which is useful in the preparation of virus samples from either fresh or frozen

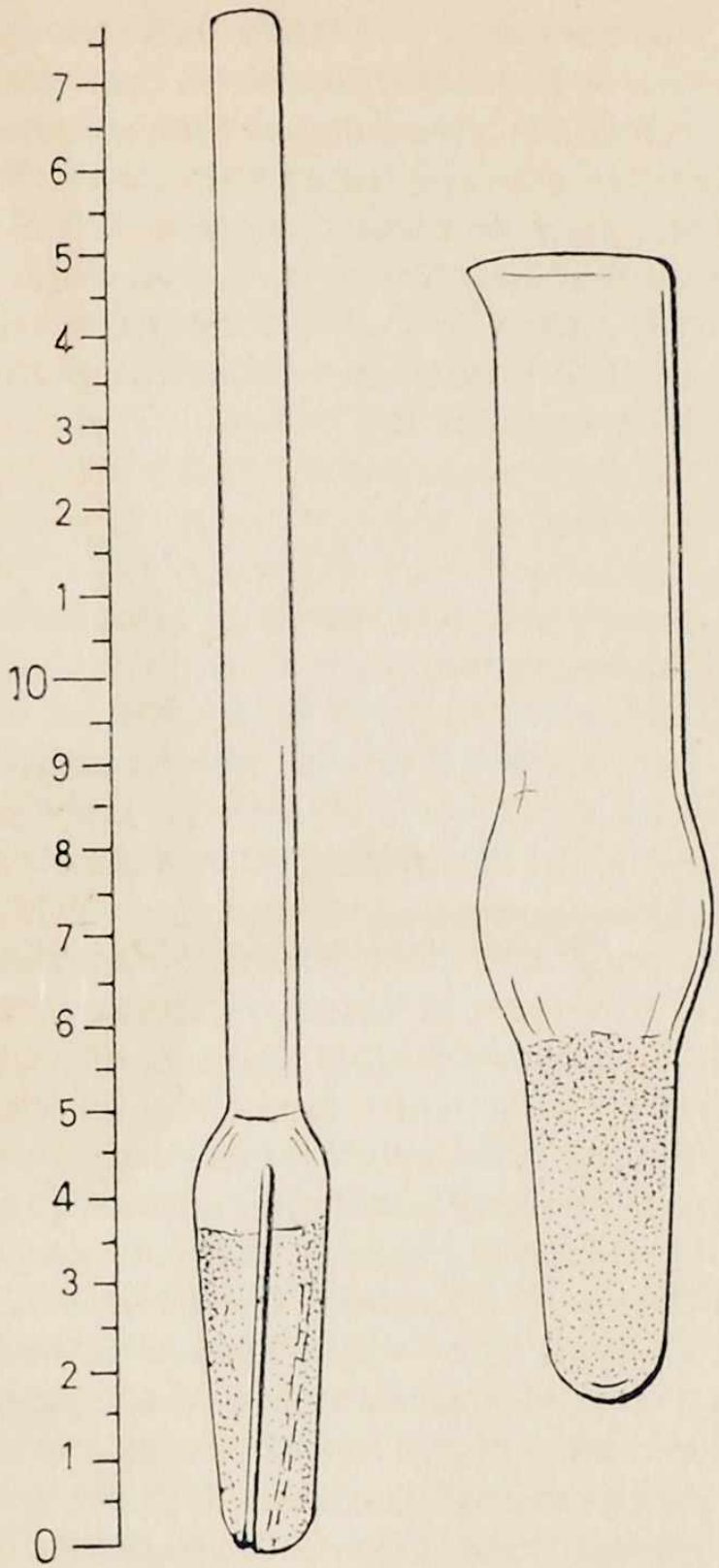
plant tissue. The two parts, the mortar and the pestle, are shown in Text Fig. 1. There are two continuous grooves cut on the grinding surface of the pestle which start from the shoulder around the base and up to the shoulder on the opposite side. These grooves serve to supply liquid to the grinding surface, and in addition act as gross cutting edges. The base of the pestle should be rounded to fit snugly into the rounded bottom of the mortar cavity; this avoids compressing the tissue into an unhomogenized mass at the bottom. The bulge on the mortar serves as a receptacle for the homogenate which can easily be reached by means of a stick tipped with cotton-wool. When necessary, the cutting surfaces may be sharpened, and at the same time fitted, by grinding with a suspension of fine emery powder.

STORAGE OF VIRUSES

A useful and simple technique for storing labile plant viruses has been devised by McKinney (1947). He considered that since many unstable viruses will retain their infectivity in expressed sap at low temperatures it seemed reasonable that some of the labile viruses will retain a large part of their activity in dry tissue. In this method leaf tissue only is used and desiccation is carried out over calcium chloride. The leaf material is shredded, without the midribs, and placed on a piece of copper gauze resting on calcium chloride in ordinary petri dishes. They are then left for three weeks, after which they can be stored in the cold room in corked tubes with a layer of calcium chloride at the bottom and a plug of muslin or cotton-wool between the specimen and the calcium chloride.

Some samples of wheat leaves infected with the virus of wheat streak mosaic, stored at 1° C. over calcium chloride, retained infectivity for 5 years (Lal & Sill, 1958).

An alternative might be the use of silica gel in the place of calcium chloride. The storage of infected leaves in polythene bags and then placing them in the 'deep freeze' works



Text Fig. 1. A ground-glass homogenizer. (*After Takahashi,* Phytopathology, *1951*)

well for stable viruses. The leaves, however, must not be allowed to dry so that polythene bags should be used. There seems to be little information on the storage of labile plant viruses by this method except for a statement by Best & Gallus (1956–7) on the storage of one of the most unstable viruses, that of tomato spotted wilt. They maintained this virus in an infective state with little, if any, loss of infectivity for periods of up to 3.6 years by storing infective tissues in sealed containers at the temperature of solid carbon dioxide (−69° C.).

CHEMICAL TREATMENT OF INOCULUM

Some viruses such as that of tomato spotted wilt are rapidly inactivated by oxidation when in expressed sap and are thus rather difficult to transmit by mechanical inoculation. Addition to the extracted sap of sodium sulphite, Na_2SO_3, by reason of its reducing action retards oxidation and prolongs the viability of the virus (Bald & Samuel, 1934; Ainsworth, 1936).

Similarly the use of phosphate by increasing the susceptibility of the leaves of beans also greatly increases the percentage of successful virus transmissions (Yarwood, 1952). However, phosphate must be used with caution when inoculating bean leaves (*Phaseolus vulgaris*) with virus. Solutions of K_2HPO_4 can be injurious and the injury increases with increasing time of exposure and concentration. The addition of 1 per cent. K_2HPO_4 to the inoculum increases the number of local lesions formed on bean leaves with the viruses of tobacco mosaic, tobacco necrosis, alfalfa mosaic, white clover mosaic, cucumber mosaic, pea mosaic and cabbage black ringspot. With other viruses and on other host plants the effect of the phosphate was much less (Yarwood, 1952).

Fulton (1957) describes a rapid method for the successful transmission of some unstable viruses from stone fruits. He obtained clear infective preparations by grinding the infected tissue with about twice its volume of calcium phos-

phate paste in 0·03M phosphate buffer and centrifuging for 1 minute or less. Infectivity of the supernatant was equal to or greater than that of untreated extracts and remained infective much longer. The method is effective with Prunus viruses A, B and H, and with the viruses of rose and apple mosaics. It was not successful with some other viruses such as those of tobacco ringspot, cucumber mosaic, tomato spotted wilt and peach yellow bud mosaic which adsorbed and sedimented with the calcium phosphate.

By combining the use of phosphate with carborundum powder the number of local lesions can be greatly increased. This is especially applicable to tobacco mosaic on the tobacco variety Xanthi-nc. Fine carborundum powder is applied evenly to the leaves by sieving through fine mesh nylon taffeta; the virus, in a phosphate buffer, is then applied by means of a stiff brush (Takahashi, 1956). Similarly Behara *et al.* (1956) obtained more local lesions with tobacco mosaic virus on Scotia bean (*Phaseolus vulgaris*) when the inoculum contained 10 per cent. 600-mesh carborundum powder with 0·1M phosphate buffer at pH 8·5 as compared with simple inoculation.

SOME ALTERNATIVE INOCULATION METHODS

Takahashi (1947) and Yarwood (1952) used a stiff poster brush for inoculation; the latter dusted the leaves with carborundum and then applied a virus-water solution to the carborundum-dusted surface by means of a brush. This method gave 828 lesions on halves of leaves inoculated with the stiff poster brush, compared to 154 on those inoculated by means of the finger. Yarwood (1957*a*) has developed this technique further into a brush-extraction method for virus transmission, a method especially suitable for plants with hairy leaves. When the stiff poster brush was stroked over the surface of a hairy leaf of a virus-infected plant and then over the leaf of a healthy susceptible plant, a high level of infection usually resulted. The method was successful with the viruses of tobacco mosaic, tobacco ringspot, tomato

spotted wilt and alfalfa (lucerne) mosaic. The use of carborundum increased the efficiency of the method in all cases. Where the leaves of the virus-infected plant were not hairy, the amount of virus obtained for inoculation could be increased if the brush was stroked sufficiently hard to break the epidermis. This method is rapid and allows the virus to be taken from either the upper or lower epidermis at will.

In cases where the virus is unstable and may be rapidly inactivated when the sap is extracted, a quick dry method of inoculation is preferable to the more conventional technique. This consists in rubbing the cut edges of leaf discs from the infected plant over the leaves of the healthy plant which have previously been dusted with carborundum or sprayed with potassium monohydrogen phosphate. One or more discs may be used at a time, the optimum being four; the discs should be put together and trimmed to a straight edge. This method has proved successful with such viruses as those of tomato spotted wilt and apple mosaic on bean (*Phaseolus vulgaris*) (Yarwood, 1953).

For large-scale inoculation it is sometimes possible to infect the plants by means of a high-pressure spraying machine containing infective sap and carborundum powder (McKinney & Fellowes, 1951).

AIDS TO INFECTION

Treatment of Plants before and after Inoculation

We are not concerned here with the effect of environmental conditions on the symptomatology which is dealt with in Chapter II, but on susceptibility to infection which may be profoundly influenced by changes in the environmental conditions, especially light, temperature and nutrition.

A most important environmental factor in the successful transmission of viruses by inoculation is the intensity of the light. Thus, reducing the light intensity under which plants are grown in summer increased their susceptibility to infection with four viruses. With tobacco necrosis and

tomato bushy stunt viruses shading of the plants before inoculation increased the average number of local lesions per leaf by more than ten times, and with the viruses of tobacco mosaic and aucuba mosaic by more than five times. Not only is susceptibility to infection enhanced but the actual virus content of the infected plant is also greatly increased. In the case of a strain of tobacco necrosis the virus content was as much as twenty times greater than that of the controls. Since tobacco necrosis virus does not become systemic in tobacco, the plants referred to here, the increase in virus content applies only to the inoculated leaves. However, increases of up to ten times in plants systemically infected with tomato bushy stunt virus were also obtained.

Furthermore, in the case of tobacco necrosis virus there was the additional advantage that the total solid content of the sap was reduced by one-half. This of course greatly facilitates the purification of the virus (Bawden & Roberts, 1947). It should therefore be remembered when plants are being raised for virus purification or local lesion work the light intensity is of great importance; since in the case of some viruses, at least, shading not only increases the number of local lesions but increases the amount of virus whilst reducing the solid content of the sap. This makes easier the purification of the virus.

Keeping the plants in complete darkness before and after sap-inoculation with tobacco mosaic virus results in a greater number of lesions on the leaves of *Nicotiana glutinosa* than on those kept in the light (Weintraub & Kemp, 1958). Not all plants of the same species react to a given virus with the same degree of susceptibility, under pre-inoculation darkening, and one particular variety of tobacco may show a greater increase in susceptibility than others (Troutman & Fulton, 1958).

On the whole it seems to be the pre-inoculation shading or darkening which consistently increases the virus susceptibility of the test plants. Post-inoculation darkening seems to have little effect or may even decrease susceptibility.

Short periods in the dark seem to be equivalent to longer periods in the shade, but it must be remembered that different plant species react differently. The optimum conditions for bean plants (*Phaseolus vulgaris*) seem to be 24 hours in the dark before inoculation, whereas witch tobacco plants susceptibility increases with increasing time in the dark up to 5 days (Bawden & Roberts, 1948).

The work of Kassanis (1952) and others suggests strongly that by exposing the experimental plants to high temperatures before inoculation greatly increases susceptibility to many viruses, although it is clear that there is great variation in the reaction of different plants to different viruses under these conditions.

Keeping the test plants at 36° C. for some time before inoculation has been shown by Kassanis greatly to increase their susceptibility to five sap-transmissible viruses, those of tomato spotted wilt, tobacco mosaic, tobacco necrosis, tomato bushy stunt and cucumber mosaic. The effects of post-inoculation treatment, however, differed with the various viruses according to whether they have high- or low-temperature coefficients of thermal inactivation. The viruses of tomato spotted wilt and tobacco mosaic can multiply in plants at 36° C., and post-inoculation treatment reduced the number of local lesions formed to between 10 and 90 per cent. of the control. On the other hand, the viruses of tobacco necrosis, tomato bushy stunt and cucumber mosaic, which do not multiply in the plant at 36° C., failed to form any local lesions at all when the plants were given post-inoculation heating.

Kassanis (loc. cit.) describes a simple glass incubator for heating the plants which was kept in the glasshouse. The incubator consisted of a metal frame 45 cm. high, 60 cm. wide and 90 cm. long, had a metal floor and glass sides and top. It was not airtight as the glass panels did not fit tightly to the frame. It opened from the top, and the four 230-W. electric heaters were fixed two on each of the longest sides, near the sides and 12 cm. from the floor. The temperature was controlled by a thermostat, the sensitive element of

which was inserted through a side wall and reached the centre of the incubator. A thermometer was placed close to it. Ten small or eight large (5 and 7 in. in diameter) pots with plants could be treated at a time; they stood in a trough of water, which occupied the entire floor of the incubator. The thermostat was adjusted to 36° C. and the temperature in different parts of the incubator usually did not vary beyond the range of 35–37° C.

If, as seems probable, it is the physiological condition of the plant which is affected by the high temperatures, then one would expect to get varying results according to the type of test plant used. Plants of bean, *Phaseolus vulgaris*, become more resistant to infection with the virus of alfalfa mosaic if exposed either to low or high temperatures before inoculation. On the other hand, they become more resistant to tobacco mosaic virus if exposed to low rather than moderate or high temperatures before inoculation. The resistance imposed by temperatures was readily lost by the plants (Panzer, 1958).

Yarwood (1952) has shown that the susceptibility of test plants to virus infection can be greatly increased by applying the pre-inoculation heat to the plants in a different way. He actually immersed the plants in hot water for periods. When primary leaves of bean were heated 3–24 hours after inoculation, the number and size of local lesions resulting from inoculation with tobacco mosaic virus or with apple mosaic virus were increased as much as twenty times in comparison with unheated leaves. When bean leaves, var. Pinto, were heated by dipping in water at 50° C. for 25 seconds, 6 hours after inoculation, the greatest increase in numbers of lesions resulted. Post-inoculation heating, however, was less effective than pre-inoculation heating for increasing the number of tobacco mosaic virus lesions. Post-inoculation heating of beans inoculated with alfalfa mosaic virus delayed the appearance of local lesions, but when they appeared they were larger and more numerous than on the unheated leaves. Heating cucumber plants, which had passed the age of greatest susceptibility, for

about 20 seconds at 50° C. as late as 5 days after inoculation with the viruses of alfalfa mosaic, apple mosaic, tomato spotted wilt, and peach yellow bud mosaic increased the number of systemically infected plants.

VIRUS INHIBITORS

One of the puzzling phenomena which confronts the student during inoculation studies with sap-transmissible viruses is the non-transmissibility of such viruses from certain plant hosts. This is usually due to the presence in the sap of the virus-infected plant of a substance which prevents infection with the virus when rubbed on the leaves of a susceptible host. Such a substance is known as an 'inhibitor'; these inhibitors are not present only in the sap of certain plants; a wide range of miscellaneous substances also have the same inhibitor effect. In this discussion, however, we are mainly concerned with the inhibitors which occur naturally in plant sap and with which the student will come in contact during the study of sap-transmissible viruses. For a more detailed account of inhibitors from other sources and for a review of the whole subject the reader is referred to Bawden (1954).

The first suggestion of an inhibitor in plant sap occurs in the work of Allard (1914, 1918), who worked with a mosaic disease of pokeweed, *Phytolacca decandra*. He was able to transmit the virus mechanically from pokeweed to pokeweed but not to tobacco. Later, Doolittle & Walker (1925) transmitted the same virus from pokeweed to healthy cucumber plants by means of aphids and showed the virus to be that of cucumber mosaic. They, also, failed to infect cucumber by mechanical inoculation. In the same year Duggar & Armstrong (1925) experimented with the sap of *Phytolacca* and found that when added to sap containing tobacco mosaic virus it prevented the infection of healthy tobacco plants. These workers tested a number of other plants for the presence of inhibitors and found that the sap

of the thorn apple *Datura stramonium* was also somewhat inhibitory.

Grant (1934) made a series of tests for the presence of inhibitors in the sap of different plant species and found that the juice from spinach, *Spinacia oleracea*, beet (*Beta vulgaris*) and Swiss chard (*B. vulgaris* var. *cicla*) all contained an inhibitory substance. It was not, however, till much later that a serious attempt was made to isolate the inhibitor from pokeweed; Kassanis & Kleczkowski (1948), by means of precipitation with alcohol, followed by adsorption on kieselguhr and elution with 10 per cent. sodium chloride, identified it as a glycoprotein containing 14 to 15 per cent. of nitrogen and 8 to 12 per cent. carbohydrate. It occurs in the sap of *Phytolacca esculenta* leaves at the rate of 100 mg./l.

Kuntz & Walker (1947) studied the inhibitory substances in spinach sap and decided that there were two inhibitors present, one of which inhibits infection of tobacco with the cabbage black ringspot virus but does not affect the transmission to tobacco of tobacco mosaic virus. This one withstands boiling, exposure to alcohol and diffuses through cellophane; the other is destroyed by heating at 70° C., by 95 per cent. alcohol and by acid pH below 3 or alkaline pH above 9·5, and does not diffuse through cellophane. This latter inhibitor may be a protein similar to that from *Phytolacca* as suggested by Bawden (loc. cit.).

Gendron & Kassanis (1954) have studied the importance of the host species in determining the action of virus inhibitors. They consider that the extent to which infection is inhibited by those substances depends on the species of plants to which inoculations are made and not on the identity of the virus. The inhibitors are largely ineffective in preventing infection of the species which contain them. For example, sap from *Datura tatula* inhibits infection when inoculations are made to bean, *Phaseolus vulgaris*, and to beet but not when made to *D. tatula*, and sap from beet inhibits infection of beans and *D. tatula* but not of beet.

There is one anomalous case which may be an exception

to this behaviour. It is extremely difficult to inoculate young plants of lovage (*Ligusticum scoticum*) with a mosaic virus from an infected lovage plant, although the virus is easily inoculated by sap to tobacco and many other plant species (Smith & Markham, 1944).

A few examples of plants which contain inhibitors of virus infection follow: dahlia mosaic virus sap-transmissible from infected to healthy *Zinnia elegans*, but not from this species to dahlia (Brierley & Smith, 1950); carnation mosaic virus from carnation (*Dianthus caryophyllus*) to carnation but rarely from carnation to bean or tobacco (van der Want, 1951); tobacco ringspot virus easily transmissible from infected to healthy sweet william (*D. barbatus*), but not from sweet william to tobacco or cucumber (Weintraub & Gilpatrick, 1952).

In experiments at Cambridge with a virus from *Ranunculus* sp. in New Zealand spinach (*Tetragonia expansa*), it was found easy to transmit the virus from and to New Zealand spinach but not from that plant to other plant species. Benda (1956) finds that expressed sap of New Zealand spinach when mixed with tobacco ringspot virus inoculated by rubbing onto cowpea (*Vigna sinensis*) leaves caused a delay in the appearance of the primary virus symptoms. The spinach sap appeared to contain two active fractions, one an inhibitor which decreased the number of lesions but was destroyed by heat, and the other an augmenter, identified indirectly as a soluble oxalate salt, which increased the number of lesions.

Healthy and infected cucumber-plant extracts have been found highly inhibitive to cucumber mosaic virus. The inhibitor is present in almost all parts of the cucumber plant with the exception of the corollas. It has been detected in green leaves, dead leaves, cotyledons, stems, etiolated seedlings, roots, seeds, fruits and entire blossoms (Sill & Walker, 1952).

An inhibitor from rice leaves which is also present in the flowers, roots, kernels, culms, polish from the rice and in heat-dried and frozen leaves, prevents the infection of

leaves of Pinto bean (*Phaseolus vulgaris*) with tobacco mosaic virus (Allen & Kahn, 1957).

In the expressed sap of various dodders (*Cuscuta* spp.) are substances inhibitory to the development of the following viruses: tomato spotted wilt virus on *Nicotiana glutinosa*, lucerne (alfalfa) mosaic virus on *Phaseolus vulgaris*, cabbage black ringspot virus on tobacco, potato virus Y on *Physalis floridana*, potato virus X on tobacco and *Amaranthus retroflexus*, cucumber mosaic virus on cowpea (*Vigna sinensis*), potato rattle virus on *N. glutinosa* and tomato bushy stunt virus on *N. glutinosa* (Schmelzer, 1956).

Bawden & Pirie (1957) have isolated a virus-inactivating system even from the leaves of tobacco itself. The exposure of stable virus preparations of the Rothamsted strain of tobacco necrosis virus to leaf-sap sediment, which had been centrifuged at 4,000 to 8,000 *g*, in the presence of air, inactivated them. This accounts for the variations in infectivity in tobacco necrosis viruses prepared from tobacco sap by different methods.

Inhibitors of a different type from the foregoing occur in a number of different plants; these are tannins and they are probably the reason for the difficulty in transmitting mechanically the viruses occurring in rosaceous plants, particularly strawberries and raspberries. When leaves, stems or roots of strawberry plants are macerated, extracted with a little water, and the extracts centrifuged, the supernatant fluid contains no protein. Enough tannin is liberated to precipitate all the plant protein and the supernatant still contains enough tannin to precipitate tobacco mosaic virus and prevent it from infecting *N. glutinosa* (Bawden & Kleczkowski, 1945). Thresh (1956) suggests methods for increasing the efficiency of virus extraction by preventing or reversing the precipitation of proteins which normally occurs on macerating tissues containing tannins. Since tannins are not uniformly distributed throughout the plant, and their concentration varies with the season, it is evident that the best inoculation results will be obtained

by selecting tissues with a high virus content and the minimum amount of tannin. In the undamaged cell, tannins are localized in the vacuole and separated from the protoplast, so that the simplest way to avoid virus precipitation is to complete the inoculation before the tannins have had time to accumulate and combine with the proteins released when the leaves are macerated. A quick method of inoculation such as Yarwood's leaf-disc method (see p. 114) is therefore to be recommended where tannins are present.

In cases where the virus is precipitated by the tannins of the host it may be possible to recover infective virus by centrifuging the expressed sap. Thresh (loc. cit.) suggests that this may be the explanation of Bennett's (1955) recovery of infective curly-top preparations from the precipitate which forms rapidly in expressed sap of the water pimpernel (*Samolus parviflorus* Raf.).

Limasset (1951) found that nicotine sulphate increased the infectivity of saps containing tannins, and Thresh showed that tannic acid in the inoculum inhibited less in the presence of nicotine sulphate. Cadman (1956) used the same substance in experiments with raspberry viruses. In extracting the virus from raspberry leaves, 40 ml. of 40 per cent. nicotine sulphate solution was applied per 30 gm. of leaf tissue.

REFERENCES

AINSWORTH, G. C. (1936). 'Detection of spotted wilt virus in Chrysanthemum.' *Nature, Lond.* **137**, 868.

ALLARD, H. A. (1914). 'The mosaic disease of tobacco.' *U.S. Dept. Agric. Bull.* no. 40.

(1918). 'Mosaic disease of *Phytolacca decandra*.' *Phytopathology*, **8**, 51–54.

ALLEN, T. C. and KAHN, R. P. (1957). 'Tobacco mosaic virus inhibition by rice extracts.' *Phytopathology* (abstr.), **47**, 515.

BALD, J. G. and SAMUEL, G. (1934). 'Some factors affecting

the inactivation rate of the virus of tomato spotted wilt.' *Ann. appl. Biol.* **21,** 179–90.

BAWDEN, F. C. (1950). *Plant Viruses and Virus Diseases.* 3rd ed. Waltham, Mass.: Chronica Botanica Co.

(1954). 'Inhibitors and plant viruses.' *Advances in Virus Research*, **2,** 31–57.

BAWDEN, F. C. and KLECZKOWSKI, A. (1945). 'Protein precipitation and virus inactivation by extracts of strawberry plants.' *J. Pomol.* **21,** 2–7.

BAWDEN, F. C. and PIRIE, N. W. (1957). 'A virus-inactivating system from tobacco leaves.' *J. gen. Microbiol.* **16,** 696–710.

BAWDEN, F. C. and ROBERTS, F. M. (1947). 'The influence of light intensity on the susceptibility of plants to certain viruses.' *Ann. appl. Biol.* **34,** 286–96.

(1948). 'Photosynthesis and predisposition of plants to infection with certain viruses.' *Ann. appl. Biol.* **35,** 418–28.

BEHARA, L., VARZANDEH, M. and THORNBERRY, H. H. (1956). 'Mechanism of the action of abrasives on infection by tobacco mosaic virus.' *Virology*, **1,** 141–51.

BENDA, G. T. A. (1956). 'The effect of New Zealand spinach juice on the infection of cowpeas by tobacco ringspot virus.' *Virology*, **2,** 438–54.

BENNETT, C. W. (1955). 'Recovery of water pimpernel from curly top and the reaction of recovered plants to reinoculation with different virus strains.' *Phytopathology*, **45,** 531–6.

BEST, R. J. and GALLUS, H. P. C. (1956–7). *Rep. Waite Agric. Res. Inst. S. Australia*, p. 19.

BRIERLEY, P. and SMITH, F. F. (1950). 'Some vectors, hosts and properties of dahlia mosaic virus.' *Plant Dis. Reptr.* **34,** 363–70.

CADMAN, C. H. (1956). 'Studies on the etiology and mode of spread of raspberry leaf-curl disease.' *J. host. Sci.* **31,** 111–18.

CHESTER, K. S. (1935). 'A serological estimate of the

absolute concentration of tobacco mosaic virus.' *Science*, **82**, 17.

DALE, J. L. (1956). 'Tobacco mosaic virus infection of bean as influenced by leaf treatments.' *Diss. Abstr.* **16**, 1566.

DOOLITTLE, S. P. and WALKER, M. N. (1925). 'Further studies on the overwintering and dissemination of cucurbit mosaic.' *J. agric. Res.* **31**, 1–55.

DUGGAR, B. M. and ARMSTRONG, J. K. (1925). 'The effect of treating the virus of tobacco mosaic with the juices of various plants.' *Ann. Missouri Bot. Garden*, **12**, 359–66.

FULTON, R. W. (1957). 'A rapid method for partial purification of some unstable plant viruses.' *Phytopathology* (abstr.), **47**, 521.

GENDRON, Y. and KASSANIS, B. (1954). 'The importance of the host species in determining the action of virus inhibitors.' *Ann. appl. Biol.* **41**, 183–8.

GRANT, T. J. (1934). 'The host range and behaviour of the ordinary tobacco mosaic virus. *Phytopathology*, **24**, 331–6.

HOLMES, F. O. (1929). 'Local lesions in tobacco mosaic.' *Bot. Gaz.* **87**, 39–55.

(1952). 'Sub-mutants from non-systemic mutant strains of tobacco mosaic virus.' *Indian Phytopathology*, **5**, 9–10.

KASSANIS, B. (1952). 'Some effects of high temperature on the susceptibility of plants to infection with viruses.' *Ann. appl. Biol.* **39**, 358–68.

KASSANIS, B. and KLECZKOWSKI, A. (1948). 'The isolation and some properties of a virus-inhibiting protein from *Phytolacca esculenta*.' *J. gen. Microbiol.* **2**, 143–53.

KUNKEL, L. O. (1934). 'Studies on acquired immunity with tobacco and aucuba mosaics.' *Phytopathology*, **24**, 437–66.

KUNTZ, J. E. and WALKER, J. C. (1947). 'Virus inhibition in extracts of spinach.' *Phytopathology*, **37**, 561–79.

LAL, S. B. and SILL, W. H. (1958). 'Stability of wheat streak

mosaic virus in desiccated tissue.' *Plant Dis. Reptr.* **42,** 226–8.

LAUFFER, M. and PRICE, W. C. (1945). 'Infection by viruses.' *Arch. Biochem.* **8,** 449–68.

LIMASSET, P. (1951). 'Quelques nouveautés sur les méthodes d'extraction de certains virus.' *Atti Soc. ital. Pat.* **2,** 911–17.

McKINNEY, H. H. (1947). 'Stability of labile viruses in desiccated tissue.' *Phytopathology*, **37,** 139–42.

McKINNEY, H. H. and FELLOWES, H. (1951). 'A method for inoculating varietal test nurseries with the wheat streak-mosaic virus.' *Plant Dis. Reptr.* **35,** 264–6.

PANZER, J. D. (1958). 'The effect of pre-inoculation temperature on test plant susceptibility to alfalfa and tobacco mosaic virus.' *Phytopathology*, **48,** 550–2.

RAWLINS, T. E. and TOMPKINS, C. M. (1936). 'Studies on the effect of carborundum as an abrasive in plant virus inoculation.' *Phytopathology*, **26,** 578–87.

SAMUEL, G. (1931). 'Some experiments on inoculating methods with plant viruses, and on local lesions.' *Ann. appl. Biol.* **18,** 494–507.

SCHMELZER, K. (1956). 'Beiträge zur Kenntnis der Virushemmstaffe in Cuscuta-Arten.' *Zbl. Bakt.* Abt. 2, **109,** 20–22.

SILL, W. H. and WALKER, J. C. (1952). 'A virus inhibitor in cucumber in relation to mosaic resistance.' *Phytopathology*, **42,** 349–52.

SMITH, K. M. and MARKHAM, R. (1944). 'Two new viruses affecting tobacco and other plants.' *Phytopathology*, **34,** 324–9.

STEERE, R. (1955). 'Concepts and problems concerning the assay of plant viruses.' *Phytopathology*, **45,** 196–208.

TAKAHASHI, W. N. (1947). 'Respiration of virus-infected plant tissue and effect of light on virus multiplication.' *Amer. J. Bot.* **34,** 496–500.

(1951). 'A ground glass homogenizer for preparing virus samples from plant tissue.' *Phytopathology*, **41,** 481–2.

TAKAHASHI, W. N. (1956). 'Increasing the sensitivity of the local lesion method of virus assay.' *Phytopathology*, **46**, 654–6.

THRESH, J. M. (1956). 'Some effects of tannic acid and of leaf extracts which contain tannins on the infectivity of tobacco mosaic and the tobacco necrosis viruses.' *Ann. appl. Biol.* **44**, 608–18.

TROUTMAN, J. L. and FULTON, R. W. (1958). 'Resistance in tobacco to cucumber mosaic virus.' *Virology*, **6**, 303–16.

VAN DER WANT, J. P. H. (1951). 'Onderzoekingen over Anjermosaiëk II.' *Tijdschr. Plziekt.* **57**, 72–74.

VAUGHAN, E. K. (1956). 'A device for the rapid removal of tannins from virus-infected plant tissues before extraction of inoculum.' *Tijdschr. Plziekt.* **62**, 266–70.

WEINTRAUB, M. and GILPATRICK, J. D. (1952). 'An inhibitor in a new host of tobacco ring spot virus.' *Canad. J. Bot.* **30**, 549–57.

WEINTRAUB, M. and KEMP, W. G. (1958). 'The effect of darkness on lesion production in *Nicotiana glutinosa* L.' *Canad. J. Bot.* **36**, 455–6.

YARWOOD, C. E. (1952). 'The phosphate effect in plant virus inoculations.' *Phytopathology*, **42**, 137–43.

(1953). 'Quick virus inoculation by rubbing with fresh leaf discs.' *Plant Dis. Reptr.* **37**, 501–2.

(1955*a*). 'Deleterious effects of water in plant virus inoculations.' *Virology*, **1**, 268–85.

(1955*b*). 'Mechanical transmission of an apple mosaic virus.' *Hilgardia*, **23**, 613–28.

(1957). 'Mechanical Transmission of Plant Viruses.' *Advances in Virus Research*, **4**, 249.

(1957*a*). 'A brush extraction method for transmission of viruses.' *Phytopathology*, **47**, 613–14.

(1957*b*). 'Heat activation of some virus infections.' *Phytopathology* (abstr.), **47**, 38.

CHAPTER IX

Testing for Viruses

INDICATOR PLANTS

Since the very early days of plant virus study, virologists have sought for an alternative host plant when studying new or undescribed viruses, especially those which are sap-transmissible. The ideal alternative host is one which reacts promptly and characteristically to sap-inoculation, preferably with the formation of local lesions on the inoculated leaves (see Chapter X). Such a plant has become known as a 'differential host' or 'indicator plant'.

The uses of indicator plants are many and they have become an important tool in plant virus research. First, they may be used to confirm the presence of a virus in another plant which has vague or indeterminate symptoms. Secondly, they are invaluable for detecting latent virus infections; viruses which may be carried without symptoms by some plants often produce overt symptoms when transmitted to another plant. Thirdly, they can be used to indicate the presence of virus complexes and to separate the component viruses. Plants of this type have also been called 'filter plants' (Smith, 1931) because they filter out one component of a complex. In addition, they can be used to separate strains of the same virus occurring together (Johnson, 1947; Matthews, 1949). Fourthly, by their characteristic reactions it is often possible to identify a virus which may be well known already but may be effectually disguised by its infection of an unusual or uncommon host plant; and fifthly, a good indicator plant allows the quantitative study of a virus by virtue of its

formation of local lesions without systemic spread. Sixthly, by the capacity of indicator plants to reveal latent infections they have come to play an important part in horticulture and in agriculture in what is known as the 'indexing' of crops. By this means it is possible to gain an idea of the percentage of virus infection in a given crop, and such indexing is particularly useful in testing potatoes and stone-fruit trees from the presence of latent virus infections.

James Johnson (1925), one of the pioneers of plant virus research, was also one of the first to use an indicator plant. He inoculated young tobacco plants from apparently healthy potato plants and found that they became infected with what was at first known as the 'healthy potato virus' but which is now universally known as 'potato virus X'. The tobacco plant, together with *Datura stramonium*, was also used extensively at Cambridge in the early work on potato viruses. Tobacco seems to be susceptible to more viruses than any other known plant, and in plant virology it occupies a place comparable to the small 'laboratory animal' of those concerned with viruses of the higher animals.

There is now in use in plant virology a very large number of indicator plants, some of which have been developed or evolved for detecting a particular virus, whilst others are general-purpose plants and react to inoculation with many different viruses.

An ideal indicator plant is one which is easily and rapidly grown, has large leaves suitable for inoculation and, above all, reacts with the formation of local lesions on the inoculated leaf (Plate VII). For most purposes it is better that systemic infection should not follow.

One of the earliest indicator plants, after the tobacco plant, to be used was a related species, *Nicotiana glutinosa*, and it is especially suitable for use with tobacco mosaic virus. It reacts with clear, discrete local lesions which are easily countable and do not spread and run together. Its place is gradually being taken now by varieties of tobacco, *N. tabacum*, which have been specially bred for

this work. The gene governing the localization of the virus in *glutinosa* has been transferred to the tobacco plant and special varieties of *tabacum* such as *Xanthi* are much in use. In North America the bean (*Phaseolus vulgaris*), vars. Pinto, Golden Cluster and others, is also much used with tobacco mosaic virus. In England, beans are not suitable in work with this virus as the English varieties rarely, if ever, react with local lesions.

For some reason the best 'general purpose' indicator plants are found in the Chenopodiaceae, the first of these to be used being *Gomphrena globosa*, which was found by Wilkinson & Blodgett (1948) to be an excellent indicator for potato virus X. Another is *Chenopodium amaranticolor*, first used by Bennett in California and developed in this country by Hollings (1956, 1957). This plant reacts with local lesions to many viruses and it is not always easy to differentiate the various lesions and to correlate them with the causative virus. In Plate VII are given a selection of pictures of local lesions on *C. amaranticolor* caused by some common viruses, and in the Appendix is a list of some of the more well-known indicator plants and the viruses for which they can be used.

It may be helpful here to give some details of indicator plants which have been specially developed, or discovered as suitable, for a particular virus or group of viruses. In some cases it is only one variety of a plant species which can be used as an indicator.

Potato Viruses

Potato Virus A. Plants of *Solanum demissum* react to inoculation with this virus with small bluish-black local lesions (Webb & Buck, 1955). Unfortunately there is frequently a second potato virus, usually virus X, associated with virus A, and this makes diagnosis difficult since it is not always possible to differentiate between the two types of lesion. A local-lesion test for virus A in the presence of virus X has been used by Raymer & Milbrath (1957). Köhler's 'A6' virus-free clone of *Solanum*

demissum × *S. tuberosum* var. *aquila* gives somewhat similar lesions on inoculation with potato viruses A and X alone or together. However, if inoculated with virus X and allowed to become systemically infected, the plants from the resulting tubers form no lesions on inoculation with virus X, but with virus A alone or combined with virus X they form lesions characteristic of virus A.

Potato Virus S. This virus is widespread in many potato viruses, but the symptoms on potatoes are very slight and can easily be overlooked or mistaken for the normal effects of maturity. Bagnall & Larson (1957) have found the following plant species develop characteristic symptoms when inoculated with potato virus S and held at 20° C.: *Chenopodium album*, *Cyamopsis tetragonobola*, *Nicotiana debneyi*, *Saracha umbellata* and *Solanum rostratum*.

Loughnane (1957) has shown that the annual beet, *Beta macrocarpa*, is a reliable indicator plant for potato virus S. When inoculated, using a fine abrasive, and with the plants held at 60–70° C., symptoms develop on the inoculated leaves in about 20 days. Symptoms may vary and range from pale yellow spots about 2 mm. in diameter to spots about the same size which later become necrotic. If *B. macrocarpa* leaves, infected with virus S, are kept in the dark for about 48 hours, then killed in boiling water and decolorized in alcohol, the spots remain pale but each is surrounded by a thick band from which the chlorophyll is not removed by the alcohol.

Potato Virus Y. Various indicator plants reacting with local lesions have been used by different workers. Of these *Physalis floridana* and *Lycium rhombifolium* are probably the most satisfactory.

Potato Aucuba Mosaic Virus. Pepper plants (*Capsicum annuum*) have been used by Maris & Rozendaal (1956), whilst Hollings (1957) has obtained good local lesions on *Chenopodium amaranticolor* with potato aucuba mosaic virus.

Cucumber Mosaic Virus

Some varieties of bean, *Phaseolus vulgaris*, give small local

lesions when inoculated with this virus, as does also *Chenopodium amaranticolor*. In America, *C. hybridum* is recommended as a good local lesion host; necrotic local lesions develop 7 to 10 days after inoculation, using carborundum. Lesions appear as small water-soaked spots which become necrotic and enlarge to diameter of about 4 mm. (Roberts, Wilkinson & Ross, 1951). The seeds of *C. hybridum* do not germinate easily and the recommendation given by Rochow (1959) is to soak the seeds in concentrated sulphuric acid for 3–5 minutes, and then to leave under a dripping tap for several days.

Carnation Viruses

A rapid method of indexing carnation plants for viruses (ringspot, mottle and vein mottle) is to slit the tip of a shoot vertically or scrape the epidermis and wipe the cut surfaces gently over the leaves of *Chenopodium amaranticolor* dusted with 400-mesh carborundum. Local lesions develop in a few days. This method is claimed by Hollings (1957) to avoid the action of the inhibitor present in carnation sap and to be much quicker and more sensitive than the conventional method of inoculating to *Dianthus barbatus*.

Sweet Potato Internal Cork Virus

A sweet potato seedling, clone VII, which was derived at Beltsville, Maryland, from open pollinated Porto Rica seed from Louisiana, was found to be an excellent indexing host. Whatever the method used for transmission, whether mechanical, graft, or insect vector, chlorotic spots developed on the leaves in about 7 days after infection (Hildebrand, 1957).

Brome Mosaic Virus

Brome mosaic virus consistently induced distinct necrotic local lesions on leaves of *Chenopodium hybridum*. When carborundum-dusted leaves were inoculated, about twenty times as many lesions developed as when no abrasive was

used. Brushing inoculum on leaves produced more lesions than other rubbing methods. More lesions resulted from neutral 0·1M phosphate buffer as diluent than from other diluents tested, but partly purified virus preparations stored at 3° C. in neutral phosphate buffers were less infectious than similar preparations stored in distilled water or in 0·1M acetate buffer, pH 5·1. Lesion numbers were essentially inversely proportional to dilution of inocula. Inocula differing in virus content by 20 per cent. or more showed statistically significant differences in lesion numbers when assayed on 36 opposite half-leaves. The data show that plants of *C. hybridum* are reliable for local-lesion assay of brome mosaic virus (Rochow, 1959).

Hydrangea Viruses

Plants of hydrangea are liable to be infected with two ringspot viruses, those of tomato and hydrangea ringspot. *Gomphrena globosa* reacts to the former virus 6 to 10 days after inoculation with solid grey leaf spots which become bordered with red in another 5 to 6 days. Tip-killing sometimes occurs after 28 days. It is readily distinguished from hydrangea ringspot virus by its capacity to invade *Gomphrena* systemically and to infect tobacco (Brierley, 1956).

Stone-fruit Viruses

The Shirofugen variety of *Prunus serrulata* has proved to be a very valuable indicator host for the ringspot virus complex in cherries. A necrotic localized lesion with gumming occurs round each inserted bud if infected (Fig. 17) (Moore & Keitt, 1949).

Fink (1959) has indexed sour cherry trees for necrotic ringspot virus on excised twigs.

Growth of excised dormant twigs is stimulated by 3 p.p.m. gibberellin solution. A bud known to be infected with peach necrotic ringspot is grafted between two axillary buds on the twig to be indexed. If symptoms develop as the buds unfold the tree is free of ringspot; absence of symptoms indicates infection. Alternatively, a

Fig. 13. Chrysanthemum aspermy' virus

Fig. 14. Tobacco necrosis virus

Fig. 15. Tomato bushy stunt virus

Fig. 16. Cabbage black ringspot virus

Local lesions caused by different viruses on Chenopodium amaranticolor

Testing for cherry ringspot virus

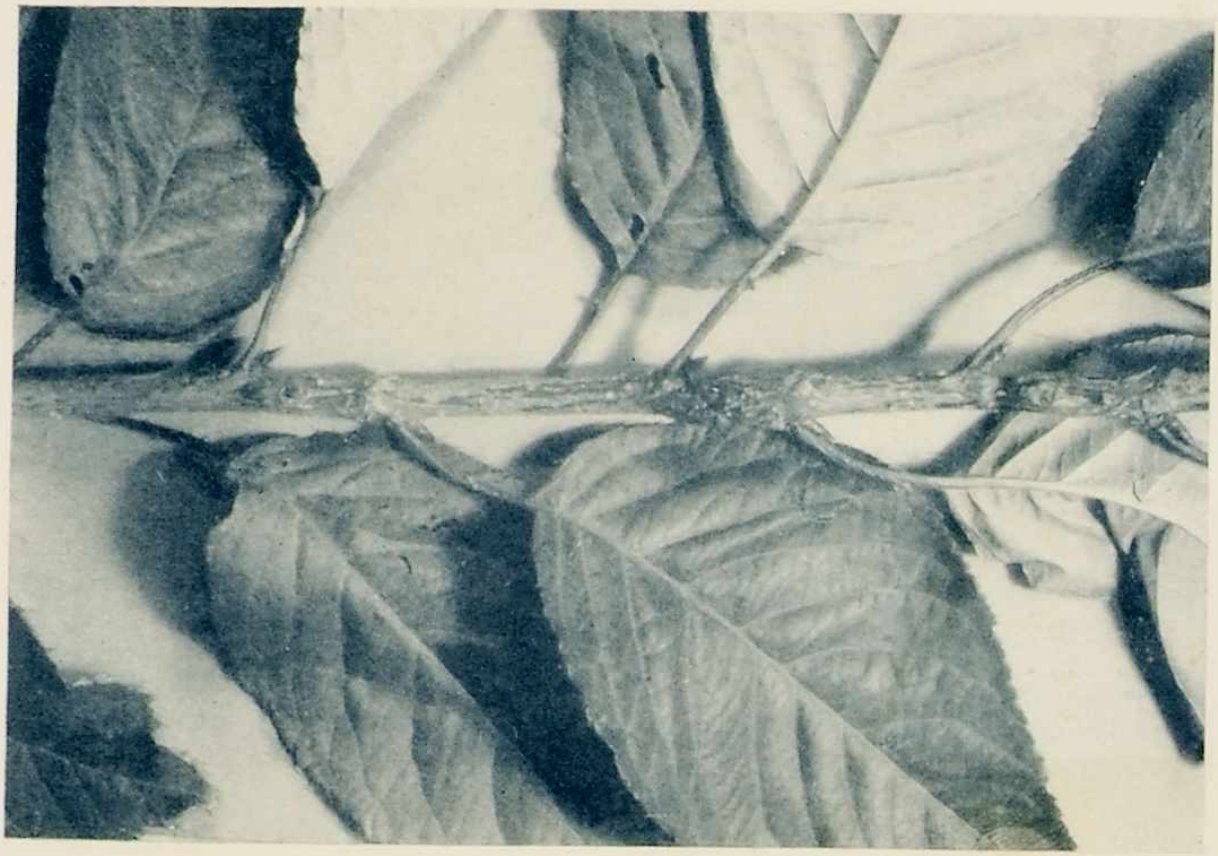


Fig. 17. Right-hand bud positive showing oozing of gum round the inserted bud, left-hand bud negative

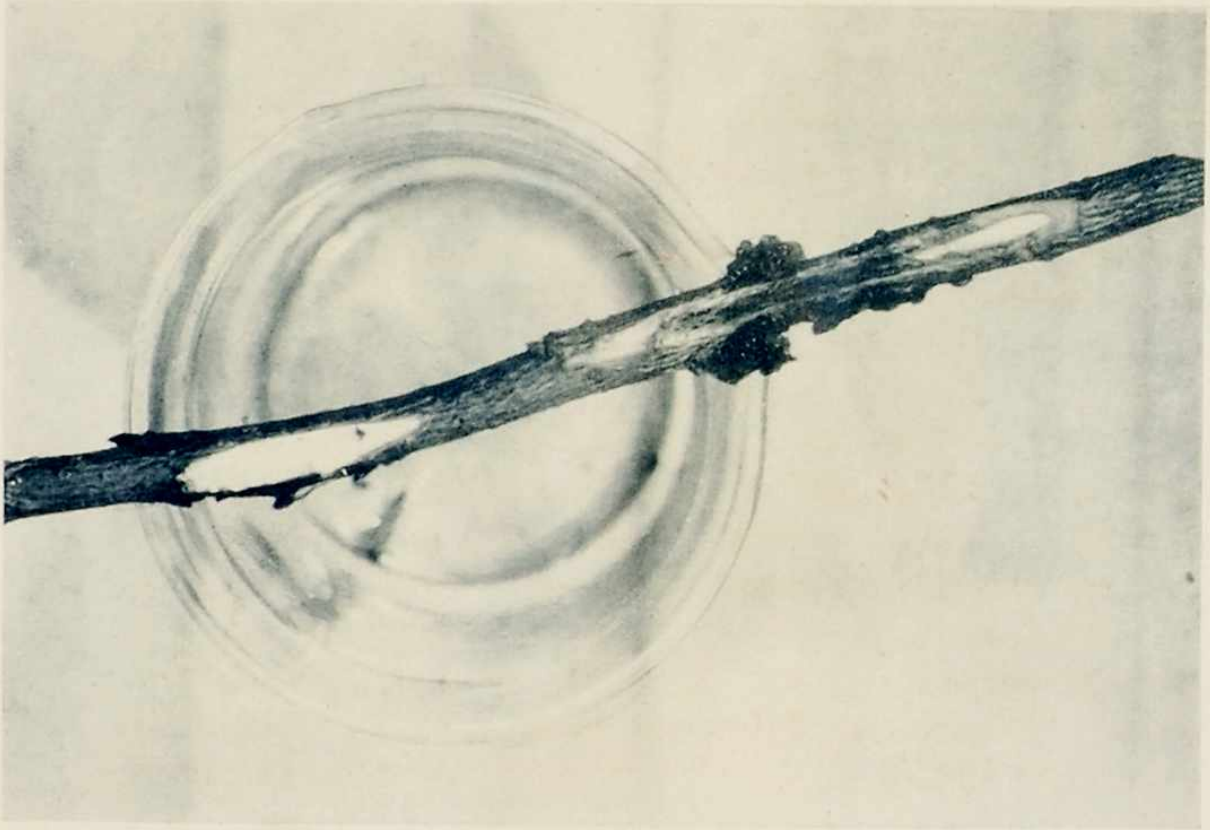


Fig. 18. Tissue cut back to the wood at the right-hand bud showing necrosis at the positive reactor, clean wood at the negative reactor at left. (After D. Cation)

bud to be indexed is grafted to a twig known to be uninfected. Appearance of symptoms on the unfolding 'known' buds indicates that the 'unknown' was infected. To index large trees bud sticks from more than one part of the tree, depending on its size, should be used.

Herbaceous plants such as the cucumber and cowpea are being developed as indicator hosts for stone-fruit viruses (Thornberry, 1957), but infections have been few and erratic. Milbrath (1953) achieved greater success by using the tip leaves and flower petals as inocula from Montmorency cherries suspected of containing latent infections. By this means he avoided the inhibitors present in the leaves of cherry trees.

The squash has been used as a differential host for strains of stone-fruit ringspot viruses. Symptom expressions vary from local lesions followed by secondary necrosis to bright golden-yellow leaf patterns. The squash variety 'Buttercup' makes a good indicator because of the bright yellow patterns which develop when the inoculum is from a tree with sour cherry yellows (Milbrath, 1956).

One particular variety of cowpea, *Vigna sinensis* var. California Blackeye No. 5, reacts with necrotic local lesions when inoculated on the primary leaves from cherry virus sources. Other varieties of cowpea do not apparently give local lesions (Thornberry, 1957).

One-year-old seedlings of *Prunus tomentosa* are recommended also as indicator plants for sour cherry viruses. This species is said to be an improvement on Montmorency cherry or Elberta peach as indicators (Fink, 1955).

Hollings (1959) has made host range studies with fifty-two plant viruses. The reactions of fifteen plant species in families related to the Caryophyllaceae when mechanically inoculated with sixty-eight isolates of fifty-two plant viruses are described. No general relationship could be detected between susceptibility to particular viruses and taxonomic relationships of the host plants. Some of the species were susceptible to many more viruses than were others. This was particularly true of *Chenopodium amaranticolor*,

Gomphrena globosa and *Tetragonia expansa* which react with many viruses in such a way as to make them suitable indicator plants; a fact made plain in this chapter.

Amaranthus caudatus, *Celosia argentea*, *Beta vulgaris* and *Spinacia oleracea* had a limited use in diagnostic or quantitative work. *Dianthus barbatus* and *Primula malacoides* were convenient plants for maintaining stock cultures of some viruses. *Phytolacca americana*, *Stellaria media*, *Fagopyrum esculentum* and *Plantago lanceolata* had little use, and *Portulacca oleracea* and *Lythrum salicaria* were useless as test plants.

All fifteen species contained inhibitors of infection; these did not prevent infection on the fifteen species, but many did so in solanaceous and leguminous plants.

DIAGNOSTIC TESTS FOR THE PRESENCE OF VIRUSES

Fruit Tree Viruses

For many years it has been the aim of plant virologists to find an easy and rapid test, other than inoculation to indicator plants, which would confirm a provisional diagnosis or reveal the presence of a latent infection. A rapid colour change is a convenient reaction, and one of the earliest instances of this is the test for phony disease of the peach. The test consists of immersing a transverse section of root in absolute methyl alcohol which has been acidified by the addition of a few drops of concentrated hydrochloric acid. After the lapse of 3 to 5 minutes the presence of phony disease is indicated by the appearance in the wood of numerous purplish spots (Hutchins, 1933). This test was used mainly for confirming a preliminary diagnosis of phony disease, but it can also be applied to the detection of the virus in apparently normal peach trees. In an examination of 191 apparently normal Elberta peach trees, 145 gave a negative reaction. Of the others, all trees whose roots in the absence of visible necrosis showed a colour reaction of two or more dots, not centrally or con-

centrically located, became phony. Other acids, organic and inorganic, produced the same reaction. Trichloracetic acid in methyl alcohol was a particularly good reagent (KenKnight, 1951). As an alternative, 2 per cent. phloroglucinol in methyl alcohol is said to give more apparently positive reactions than does the acid test solution. On wood cross-sections the phloroglucinol solution frequently develops red dots in the wood, indicating a positive reaction, up to a few centimetres beyond the point where the wood ceases to react to the acid solution. On tangential wood sections the phloroglucinol develops more striking colour reactions and gives more apparently positive reactions than the acid solution. It appears, however, as if this test is less specific as apparently positive results were obtained on tangential wood sections of peach and plum (*Prunus angustifolia*) affected with peach rosette virus. The acid test gave no reaction in these cases (KenKnight, 1952).

Another method which is applicable only to those plants which have a polyphenol system similar to that present in deciduous fruit trees consists in staining phenolic compounds, distributed in patterns, characteristic for a given virus, in cleared leaf tissue. The procedure can be used for whole leaves or sections of leaves, petioles, stems and roots. The chlorophyll is removed and the polyphenols fixed by boiling under a condenser or heating in a water bath at 80° C. in a solution of 700 ml. of 95 per cent. ethyl alcohol, 20 ml. of 37 per cent. formaldehyde, and 230 ml. of distilled water. One or two changes of the reagent may be necessary. The samples are transferred to normal sodium hydroxide and heated at 80° to 100° C. until maximum deep blue colour develops, usually from 2 to 10 minutes. The blue colour oxidizes to red in 5 to 10 minutes when exposed to air or changes to red on acidification (Lindner, Kirkpatrick & Weeks, 1950).

Potato Viruses

The phloroglucinol test has also been used for diagnosing the presence of potato leaf-roll virus in the following

technique. A piece is taken from near the base of a main stem extending from about 1 in. below soil level to above the sixth node above ground; it is trimmed off and sectioned into smaller pieces. These are cut by hand through the nodes and put into the phloroglucinol solution (1 per cent. in 50 per cent. alcohol) on a slide for one minute; the solution is then drained off and replaced for one minute by 50 per cent. hydrochloric acid. This is in turn drained off, the section mounted in water and examined under the microscope at about ×100. In a healthy plant the xylem will appear purplish-red, while all other tissues will be colourless, except some of the phloem fibres which may be pink or red. In a leaf-roll infected plant, on the other hand, some of the primary strands will be of a yellowish-red. It may be expected that if leaf-roll is present phloem necrosis will be detected in some of the six nodes under examination. It is suggested that in examining an unfamiliar potato variety, a healthy stem and, if possible, also one known to be infected with leaf-roll should be examined for comparison (Sheffield, 1943).

A modification of this technique has been suggested and found successful for diagnosing the disease under conditions of masking in the field. Concentrated hydrochloric acid (10N) replaces the 50 per cent. acid recommended by Sheffield (Wilson, 1948).

The phloroglucinol test has also been applied with success, in some potato varieties, to plants growing in the field. Necrotic flecking of the phloem, difficult to detect in the apical portion of the shoot, was rendered visible as a red discoloration. Necrosis occurs in all cases of chronic infection and most abundantly in the stem base. The use of this test on the stem apex makes it possible to determine leaf-roll increase as the season advances, as frequent sampling can be made without destroying the plant (Klostermeyer, 1950).

A technique for diagnosing potato leaf-roll virus has been developed by Sardiña *et al.* (1957).

A portion of the heel end of the potato tuber is cut out

with a cork borer to a depth of about 1 cm. Hand sections are made in the zone where vascular bundles spread in the tuber. Two sections from this zone are taken, stained for 10 minutes in 1 per cent. aqueous solution of lacmoid, rinsed in water, differentiated for one minute in 0·5 per cent. aqueous solution of acetic acid and mounted in glycerine.

In leaf-roll infected tubers thick blue callus sieve plates are observable. These plates are advanced into the sieve tubes in the form of callus plugs which, because of their abundance, give to the section a picture quite different from that shown by sections from leaf-roll-free tubers, in which only some blue sieve plates are observed. The xylem always shows a red-violet colour.

As an additional test, phloem necrosis may be demonstrated in the sieve tubes by staining the sections with phloro-glucinol-hydrochloric acid.

Another technique known as the 'colloidal precipitation method' has been used for diagnosing the presence of leaf-roll or crinkle viruses in potato tubers. The best results are obtained by mixing 1 ml. of undiluted juice with 1 ml. 0·1 per cent $HgCl_2$ and observing the precipitation after 1 hour at 20° C. Precipitation occurs with infected tubers long before it does with healthy tubers and is thought to be due to differences in protein content. The same test has been applied to the virus diseased roots of radish, turnip and sweet potato (Hirata, 1955).

In the 'iodine test', as used by Hirata (1950), a mixture of 0·5 ml. of potato tuber juice, 0·5 starch paste (1·5 gm. to 100 ml. distilled water), and 1–5 ml. iodine solution (2 gm. in 100 ml. 30 per cent. ethanol) are kept at about 30° C. The juice from tubers infected with virus faded within 50 minutes, while that from healthy tubers took more than 3 hours to fade. The diagnostic accuracy of this test seems much greater with tubers newly harvested and is much less with sprouted tubers. Gigante (1957) has applied this test successfully to demonstrate the presence of leaf-roll virus in Majestic and Sieglinde tubers and of rugose mosaic

virus in Allerfrüheste tubers. He considers, however, that the method is still open to improvement.

The fact that the tryptophane content of virus-diseased potato tubers is a great deal higher (119 per cent.) than that of healthy tubers has led to the development of a rapid test based on a quantitative xanthoproteic reaction for the differentiation of healthy and infected material. Fresh potato pulp (5 gm.) is left to stand in 40 ml. of pure 25 per cent. nitric acid for 10 minutes and then heated for 5 minutes. After cooling with running water the liquid is made up to 50 ml. with more nitric acid and filtered under pressure. The clear filtrate is gauged against a tryptophane standard in a microcolorimeter. By this means it is said that the presence in the tubers of A + X, Y + X and leaf-roll viruses can be diagnosed in 15 to 30 minutes.

Since this test is non-specific it can be used particularly in building up stocks of virus-free seed potatoes or in plant-breeding work (Schuphan, 1950).

Sugar Cane Ratoon Stunt Virus

A chemical test for the diagnosis of ratoon stunting disease of sugar-cane has been described by Farrar (1957). When 3 per cent. hydrogen peroxide is applied for 10 to 15 seconds to longitudinal sections cut from the periphery of mature basal nodes of sugar-cane, blotted off and followed by concentrated hydrochloric acid, a blue-green colour appears after 20 to 30 seconds at leaf-scar-bud level in the tissues round the vascular bundles in healthy canes. In canes affected by ratoon stunting this does not occur, or if it does, the colour is limited to one or two bundles.

There is also a natural colour reaction in the ratoon stunting disease, a pale salmon-pink blush on the immature nodes near the growing-point. It can be seen in bright light immediately after cutting primary shoots, especially of poorer stools, and may be found when these shoots are still below ground (Hughes, 1955).

Some Miscellaneous Tests

A non-specific colour test for detecting viruses in plants

has been developed at Beltsville, Maryland. When infected and virus-free stem sections, at least 1 mm. thick, are placed in 0·5 to 1 per cent. aqueous 2, 3, 5-triphenyl tetrazolium chloride, they change in colour from green to rust, then amber, and finally blood-red. The change is much more rapid in infected tissue, with a maximum difference at 35° C. within 15 to 30 minutes of immersion. A 'colour index' can be obtained by assigning the numbers 0, 1, 2 and 3 to the different colour stages and by multiplying the numbers of sections of a specific colour by the corresponding colour number (Beal, Preston & Mitchell, 1955).

The ultra-violet absorption spectra have been used as a tool for diagnosing plant virus diseases. Characteristic absorption curves for nucleic acid or its hydrolysis products were obtained for leaf tissue considered to be healthy, the height of the absorption peak at 260 mμ being a measure of the amount of nucleic acid present. The ringspot-type viruses from stone fruits produced a distinctive curve with a peak at or near 270 mμ, while those for sour cherry yellows and peach western X-disease were at or near 180 mμ. Stone-fruit viruses were detected by this method in cucumber cotyledons 48 hours after inoculation (Lindner, Kirkpatrick & Weeks, 1952).

Hirai (1956) has applied methods of paper electrophoresis to the investigation of a number of crude plant virus preparations. He finds that proteins from virus-infected plants move less readily on the filter paper than those from healthy ones; the latter move toward the cathode, the former toward the anode, and the patterns differ.

VIRUS STAINING TECHNIQUES

In this chapter a short account is given of various methods of selective staining of viruses in infected plant tissues.

Bald (1949) devised a technique of fixing and staining sections of tobacco leaves infected with tobacco mosaic virus. He used mainly epidermal strips from infected and

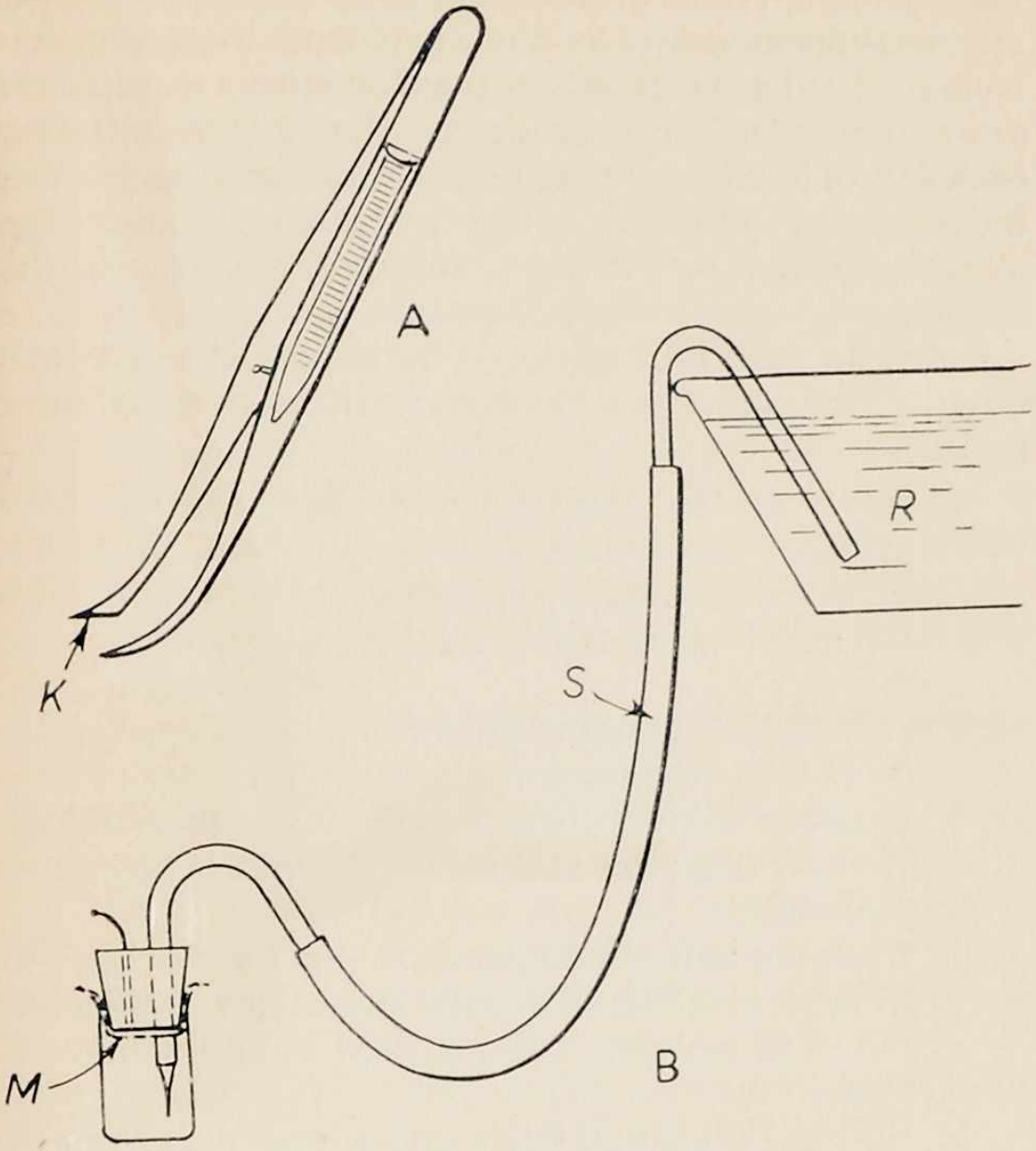
healthy tobacco plants. The epidermal strips were taken from the undersides of leaves, or from petioles or stems. The instrument used was a pair of stainless-steel forceps with smooth curved points. The lower outside edge of the arm farther from the operator was ground to a knife-edge and slightly turned so that it fitted smoothly against the curved edge of the nearer arm (Text Fig. 2). The knife-edge was pushed into the junction of two veins on the underside of the leaf or under the epidermis, the forceps closed, and a piece of the epidermis gently stripped off. The epidermal strips were placed immediately in fresh fixative contained in small glass vials.

The fixing solution was made up as follows: Lugol's iodine (6 gm. potassium iodide, 4 gm. iodine, 100 ml. water), water 25 ml., absolute alcohol, 50 ml., and formalin (38–40 per cent. formaldehyde) 5 ml.

Staining Schedule

1. Fix	30 minutes or longer
2. Wash in sodium thiosulphate solution (0·25 per cent. in 50 per cent. alcohol)	10 minutes
3. 50 per cent. alcohol, two changes	10 minutes
4. 70 per cent. alcohol	5 minutes
5. 95 per cent. alcohol	20 minutes
6. Giemsa stain 4 parts Orange G 1 part (0·2 to 0·5 ml.)	2 minutes
7. Dilute drop by drop with an equal quantity of M/50 phosphate buffer pH 7	3 minutes
8. Rinse, Lugol's iodine 1 part in 99 parts of 80 per cent. acetone	20 seconds or less
9. Absolute acetone, three changes during	5 minutes
10. Xylol	5 minutes
11. Mount in neutral Canada balsam	

In the resulting preparations of epidermal strips the amoeboid bodies, as well as other virus inclusions, stain



Text Fig. 2. A Pair of stainless steel forceps prepared for making epidermal strips. The curved edge of one arm is ground on the outside to a knife edge (K).

B. Siphon arrangement for washing epidermal strips and other material. Tap water is siphoned from reservoir (R) through tube (S) into a vial containing the material to be washed. The outlet from the vial is a hole in the stopper, covered by muslin (M). (After J. G. Bald, Phytopathology, *1949)*

purple and the host nucleus stains greenish-blue to black. In good preparations of epidermal strips no purple-staining cell constituents seem to occur apart from those that can be interpreted as virus, except that sometimes the nucleoli are also purple. The chief danger during differentiation (step 8) is of leaching out too much stain. The more gradual the transition from stain to acetone the less the shrinkage of the epidermal strips; the more rapid, the stronger the staining.

Rawlins (1957) has developed a modification of Bald's staining technique which has been found to give consistent results.

1. Peel epidermis from tobacco leaves infected with tobacco mosaic virus, float on tap water, cut with a sharp razor-blade into pieces suitable for mounting and transfer to the fixing solution by means of a glass rod.
2. Fix in Bald's alcohol-iodine-formalin fixing solution for at least 80 minutes.
3. Wash the strips in 0·25 per cent. sodium thiosulphate in 50 per cent ethyl alcohol for 10 minutes to remove the iodine.
4. Wash the strips in 50 per cent. ethyl alcohol for 10 minutes, in 70 per cent. ethyl alcohol for 10 minutes, and in 95 per cent. ethyl alcohol for 10 minutes or longer.
5. Pour the epidermal strips into a large dish of water and leave 15 minutes, wet the supper surface of the strips with drops of distilled water.
6. Pick up a strip on a microscope slide.
7. Absorb the water around the strip on filter paper.
8. Cover the strip with several drops of undiluted Giemsa solution for about 7 minutes.
9. Wash the strips in several changes of distilled water in a 400-ml. beaker for 20 minutes. The strips will appear purple after this treatment.
10. Pour the strips into a large dish of distilled water and pick up on a microscope slide.

11. Absorb the water around the strips on filter paper.
12. Add several drops of absolute ethyl alcohol to the strip and leave for a few seconds only. Place white paper under the disposal beaker so that the colour-changes in the strip may be seen. As soon as the strip changes from a purple to a blue or green colour, quickly pour off the alcohol and add several drops of clove oil to the strip. If left in the alcohol too long the cell inclusions will become blue to green like the host chromatin or cell walls.
13. After one or more minutes pour off the clove oil and replace with fresh clove oil; continue to change the clove oil until the discarded oil shows no green colour, and the cell inclusions are well differentiated when viewed under the low power of the microscope. It may require 10 minutes or more to obtain the best differentiation.
14. Pour off the clove oil and absorb the remainder from around the strip on filter paper.
15. Flood the slide with xylol to remove the clove oil and continue to change the xylol at intervals of 10 minutes. Gently move the strip on the slide during this treatment so that the xylol removes the oil from both surfaces of the strip. Do not allow strip to become dry.
16. Pour off the xylol, absorb around the strip and mount.

The crystalline inclusions and the vacuolate X bodies stain purple, and sometimes the nucleoli also. The host chromatin and cell walls stain green to blue. The method is not very satisfactory for cells that contain chloroplasts since these also may stain purple, possibly because there is enough ribonucleic acid in the chloroplasts to account for this.

The Sakaguchi reaction for arginine has been found useful for showing the uneven distribution of virus in the cytoplasm in different areas and different tissues in the tobacco leaf. In most areas of the leaf where virus is detectable by

this reaction it seems to be most concentrated in the epidermal and hair cells.

The infected tissues are fixed in Bouin's solution and then washed thoroughly in several changes of 70 per cent. alcohol; then dehydrate and section in paraffin wax.

The following reagents are needed for the Sakaguchi reaction:

A. A 1 per cent solution of 8-hydroxyquinoline in absolute alcohol; one volume of this stock is diluted with 2 volumes of water on the day it is to be used.

B. 5 ml. of a fresh solution of $5\frac{1}{4}$ per cent. sodium hypochlorite, 38 ml. distilled water, and 7·5 ml. of 0·1N sodium hydroxide. Mix on the day it is to be used.

C. 3 ml. of distilled water, 3 ml. of 0·1N sodium hydroxide, 3 g. urea; mix until dissolved. Add 14 ml. of tertiary butyl alcohol. Keep in a dropper bottle.

The following steps are used:

1. Place sections for 5 minutes in each of two changes of xylol to remove paraffin.
2. 5 minutes in each of two changes of absolute alcohol to remove xylol.
3. Transfer to solution A for 15 minutes.
4. Transfer to solution B without draining for exactly 50 seconds, until maximum red colour develops in the virus-containing areas.
5. Wash sections with solution C from a dropper bottle for about 2 minutes to remove solution B and to stop the action of the sodium hypochlorite.
6. Run slide through two changes of tertiary butyl alcohol, 10 seconds in the first and 4 minutes in the second.
7. Flood slide with xylol for 5 minutes.
8. Remove excess xylol from slide and add several drops of mineral oil.
9. Remove any remaining xylol, add cover glass and remove excess mineral oil.

10. Cover glass may be sealed with a mixture of 80 parts by weight of rosin and 20 parts of lanoline (Rawlins, Weierich & Schlegel, 1956).

McWhorter (1940) has used trypan blue to differentiate virus inclusion bodies produced by different viruses. Tulip mosaic virus and the lily latent virus both give rise to intracellular inclusions in tulips and also produce a similar type of leaf mottling.

The inclusion body formed by tulip mosaic virus stains characteristically with trypan blue and can thus be distinguished from the inclusion bodies of lily latent virus which do not stain in this way.

The virus inclusions of the cabbage black ringspot virus can also be demonstrated in epidermal strips of turnip or *Nicotiana glutinosa* by staining with trypan blue and by means of phloxine (Berkeley & Weintraub, 1952).

Tissues of potato plants infected with potato virus Y show an affinity for dilute vital stains. The rate at which necrotic changes develop in the collenchyma, phloem and parenchyma of potato plants inoculated with different strains of potato virus Y can be determined by the use of rhodamine B, neutral red and brilliant cresyl blue at concentrations of 5 p.p.m. in distilled water. In those plants developing systemic necrosis after inoculation, relatively large quantities of the dye (rhodamine B) accumulate in the collenchyma, phloem and adjacent parenchyma before any noticeable pathological changes occur in those areas (Müller & Munro, 1956).

The intracellular inclusions produced by the broad bean mottle virus in that host can be demonstrated by means of phloxine. Strips of epidermal tissue are stained in a 0·5 per cent. solution of phloxine in distilled water; trypan blue can also be used. The inclusions seem to be confined to the chlorotic areas of the infected broad bean leaves (Rubio & van Slogteren, 1956).

Littau & Black (1952) have described some spherical inclusions (spherules) in plant tumours caused by the wound

tumour virus. Material was fixed in Flemming's fluid or Carnoy; the 'spherules' stained red with safranin after treatment with Flemming's triple stain or safranin and fast green.

REFERENCES

BAGNALL, R. H. and LARSON, R. H. (1957). 'Potato Virus S.' *Phytopathology* (abstr.), **47**, 2.

BALD, J. G. (1949). 'A method for the selective staining of viruses in infected plant tissues.' *Phytopathology*, **39**, 395–402.

BEAL, J. M., PRESTON, W. H. and MITCHELL, J. W. (1955). 'Use of 2,3,5-triphenyl tetrazolium chloride to detect the presence of viruses in plants.' *Plant Dis. Reptr.* **39**, 558–60.

BERKELEY, G. H. and WEINTRAUB, M. (1952). 'Turnip mosaic.' *Phytopathology*, **42**, 258–60.

BRIERLEY, P. (1956). 'Susceptibility of *Gomphrena* to tomato ringspot virus.' *Plant Dis. Reptr.* **40**, 667.

FARRAR, L. L. (1957). 'A chemical test for ratoon stunting disease of sugar cane.' *Phytopathology* (abstr.), **47**, 10.

FINK, H. C. (1955). *Phytopathology*, **45**, 320–3.

(1959). 'Indexing sour cherry trees for necrotic ring-spot virus on excised twigs.' *Phytopathology*, **49**, 58–59.

GIGANTE, R. (1957). 'La prove dello iodio nella identificazione dei tuberi di Patata virosati.' *Boll. Staz. Pat. veg. Roma*, Ser. 3, **14**, 147–51.

HILDEBRAND, E. M. (1957). 'Rapid symptoms in seedling VII Sweet Potato of a virus always associated with internal cork.' *Science*, **126**, 751–3.

HIRAI, T. (1956). 'The diagnosis of plant virus diseases by means of paper electrophoresis.' *Forsch. Pfl. Kr. Kyoto*, **6**, 87–96.

HIRATA, S. (1950). 'Diagnostic value of the iodine method and the phenol method for virus-infected potato tubers.' *Ann. Phytopath. Soc. Japan*, **14**, 25–28.

(1955). 'Studies on the "colloidal precipitation method"

for diagnosing virus-diseased potato, radish, turnip and sweet potato.' *Mem. Fac. agric. Miyazaki Univ.* **1**, 137–77.

HOLLINGS, M. (1956). '*Chenopodium amaranticolor* as a test plant for plant viruses.' *Plant Path.* **5**, 57–60.

(1957). 'Reactions of some additional plant viruses on *Chenopodium amaranticolor*.' *Plant Path.* **6**, 133–5.

(1959). 'Host-range studies with fifty-two plant viruses.' *Ann. appl. Biol.* **47**, 98–108.

HUGHES, C. G. (1955). 'Some recent developments in the study of ratoon stunting disease.' *Cane Grow. quart. Bull.* **19**, 27–28.

HUTCHINS, L. M. (1933). 'Identification and Control of the phony disease of the peach.' *Office State Entom. Georgia Bull.* **78**, 1–55.

JOHNSON, J. (1925). 'Transmission of viruses from apparently healthy potatoes.' *Wisc. Agric. Exp. Sta. Res. Bull.* no. 63.

(1947). 'Virus attenuation and the separation of strains by specific hosts.' *Phytopathology*, **37**, 822–37.

KENKNIGHT, G. (1951). 'The acid test for phony disease of the peach and its diagnostic value.' *Phytopathology*, **41**, 20.

(1952). 'Comparison of a phloroglucinol test with the acid one for phony.' *Phytopathology* (abstr.), **42**, 285.

KLOSTERMEYER, E. C. (1950). 'The phloroglucinol test for diagnosis of leaf-roll in Netted Gem potatoes.' *Plant Dis. Reptr.* **34**, 36–38.

LINDNER, R. C., KIRKPATRICK, H. C. and WEEKS, T. E. (1950). 'A simple staining technique for detecting virus diseases in some woody plants.' *Science*, **112**, 119–20.

(1952). 'Ultraviolet absorption spectra as a tool for diagnosing plant virus diseases.' *Science*, **115**, 496–9.

LITTAU, C. VIRGINIA and BLACK, L. M. (1952). 'Spherical inclusions in plant tumours caused by a virus.' *Amer. J. Bot.* **39**, 87–95.

LOUGHNANE, J. B. (1957). 'A necrosis of potato tubers

caused by the paracrinkle virus.' *Proc. 3rd Confer. Pot. Virus Dis.* Wageningen,' 179–83.

McWHORTER, F. P. (1940). 'Separation of Tulip I virus from lily latent virus by cytological methods.' *Phytopathology* (abstr.), **30,** 788.

MARIS, B. and ROZENDAAL, A. (1956). 'Enkele proeven met stammen van het X- en het aucubabontvirus van de aardappel.' *Tijdschr. Plziekt.* **62,** 12–18.

MATTHEWS, R. E. F. (1949). 'Reactions of *Cyphomandra betacea* to strains of potato virus X.' *Parasitology*, **39,** 241–4.

MILBRATH, J. A. (1953). 'Transmission of components of the stone-fruit latent virus complex to cowpea and cucumber from cherry flower petals.' *Phytopathology* (abstr.), **43,** 479.

(1956). 'Squash as a differential host for strains of stone fruit ringspot viruses.' *Phytopathology* (abstr.), **46,** 638.

MOORE, J. D. and KEITT, G. W. (1949). 'An indexing method for necrotic ringspot and yellows of sour cherry.' *Phytopathology* (abstr.), **39,** 15–16.

MÜLLER, K. O. and MUNRO, J. (1956). 'The affinity of potato virus Y-infected potato tissues for dilute vital stains.' *Phytopath. Z.* **28,** 70–82.

RAWLINS, T. E. (1957). 'A modification of Bald's stain for viruses and for cell-inclusions associated with virus infections.' *Phytopathology*, **47,** 307.

RAWLINS, T. E., WEIERICH, W. A. and SCHLEGEL, D. E. (1956). 'A histochemical study of certain plant viruses by means of the Sakaguchi reaction for arginine.' *Virology*, **2,** 308–11.

RAYMER, W. B. and MILBRATH, J. A. (1957). 'A local-lesion test for potato virus A in the presence of potato virus X.' *Phytopathology* (abstr.), **47,** 532.

REITER, L. (1957). 'Vitale Fluorochromierung pflanzlicher Viruseinschlusskörfer.' *Protoplasma*, **48,** 279–86.

ROBERTS, D. A., WILKINSON, R. E. and ROSS, A. F. (1951). '*Chenopodium hybridum*, a local-lesion host for cucumber mosaic virus.' *Phytopathology*, **41,** 31.

ROCHOW, W. F. (1959). '*Chenopodium hybridum* as a local-lesion assay host for brome mosaic virus.' *Phytopathology*, **49**, 126–30.

RUBIO, H. and VAN SLOGTEREN, D. H. M. (1956). 'Light and electron microscopy of X-bodies associated with broad bean mottle virus and *Phaseolus Virus 2*.' *Phytopathology*, **46**, 401–2.

SARDIÑA, J. R., ORAD, A. G. and SAN ROMAN, F. P. (1957). 'Some observations about techniques of diagnosing potato leaf-roll virus.' *Proc. 3rd Confer. Pot. Virus Dis.* Wageningen,' 59–70.

SCHUPHAN, W. (1950). 'Eine kolorimetrische Schellmethode (modifizierter Tryptophan-Schnelltest) zur Unterscheidung gesunder und viruskranker Kartoffeln (Vorläufige Mitteilung).' *Z. PflKrankh.* **57**, 408–15.

SHEFFIELD, F. M. L. (1943). 'Value of phloem necrosis in the diagnosis of potato leaf-roll.' *Ann. appl. Biol.* **30**, 131–6.

SMITH, KENNETH M. (1931). 'On the composite nature of certain potato virus diseases of the mosaic group as revealed by the use of plant indicators and selective methods of transmission.' *Proc. roy. Soc. B*, **109**, 251–67.

THORNBERRY, H. H. (1957). 'A cowpea local-lesion assay of presumably cherry ringspot virus.' *Phytopathology* (abstr.), **47**, 35.

WEBB, R. E. and BUCK, R. W. (1955). 'A diagnostic host for potato virus A.' *Amer. Potato J.* **32**, 248–53.

WILKINSON, R. E. and BLODGETT, F. M. (1948). '*Gomphrena globosa*, a useful plant for qualitative and quantitative work with potato virus X.' *Phytopathology* (abstr.), **38**, 28.

WILSON, J. H. (1948). 'The use of the phloroglucinol test for diagnosis of leaf-roll in potatoes.' *J. Austral. Inst. agric. Sci.* **14**, 76–78.

CHAPTER X

Local Lesions and Virus Complexes

QUANTITATIVE ASSAY OF PLANT VIRUSES

There are two main methods for making a quantitative assay of plant viruses. One is the serological method in which the precipitin test is used; this is briefly referred to in Chapter XI. The other is the 'local lesion' method, the outline of which is given here.

Certain host plants react to certain viruses in such a way that the virus is localized on the inoculated leaf (Fig. 1). This localization may be permanent as in the case of tobacco mosaic virus on the leaves of *Nicotiana glutinosa*, or there may be a coalescence of the points of infection as with tobacco necrosis virus on the leaves of bean (*Phaseolus vulgaris*) or systemic infection of the plant may follow as in the case of potato virus X on the tobacco plant. The usual reaction on the part of the plant in this type of infection is the development of numerous necrotic spots or rings, termed *local lesions*, on the leaf inoculated. Various types of local lesions can be seen in Plate VII. Where there is no systemic spread of the virus the use of local lesions allows the recognition of large numbers of successful transmissions on single plants. In 1929 Holmes showed that the number of lesions varied with the virus content of the inoculum; leaves rubbed with undiluted sap produced hundreds of lesions whilst leaves rubbed with a 1 : 1,000 dilution produced only a few. This allows for comparative estimates of virus concentrations. At higher concentrations of the virus there is no direct and simple relationship between the concentration and the numbers of

lesions produced, but within certain limits it is possible to tell which of two samples of virus is the more concentrated, and to gain some idea of their relative virus content. It would be quite wrong, however, as Bawden (1950) has emphasized, to translate differences in numbers of lesions directly to differences of virus content.

There are many variables to be taken into account when using the local lesion method and much work has been carried out to try and make the results statistically correct. It is important that the plants to be used should be of comparable age, size and colour, and all should have the same nutritional treatment. In using *N. glutinosa*, for example, the number of leaves should be reduced to four or five and the growing-point removed.

One of the variables mentioned above is the fact that all the leaves of the same plant do not react to inoculation in the same manner. It was shown by Samuel & Bald (1933), however, that there was little difference between the reaction of opposite halves of the same leaves, and that by comparing preparations on opposite half-leaves fewer plants could be used and more accurate results obtained. Bawden (1950) points out that the simplest way is to select one preparation as a standard and apply it to one half of every leaf, while the other half-leaves are apportioned between the other preparations. Each preparation can then be compared directly with the standard and indirectly through the standard with any other.

Youden & Beale (1934) used the Latin square to permit the intercomparison of a number of virus preparations without unnecessary duplication of a reference standard. This is accomplished by so distributing the several treatments among the leaves that each appears equally often on each plant and each leaf position. Thus, if five virus preparations are to be compared, using five plants each having five leaves, each virus preparation is inoculated on to a total of five leaves, once on each plant and once in each leaf position.

Steere (1955) suggests that one should designate a right

and a left side and inoculate the right sides of half the leaves with one preparation and the left halves of the same leaves with the second preparation. The right halves of the remaining leaves should then be inoculated with the second preparation and the left halves with the first. This will provide a balance against unequal manipulation of the two halves of a leaf.

For information on the statistical aspect of the local lesion technique the reader is referred to Bawden (1950), Kleczkowski (1949), Bald (1950) and Fry & Taylor (1954).

The number of local lesions is increased if an abrasive, celite or fine carborundum powder, is added to the inoculum. Great care, however, must be used or the leaf will be damaged; it is important, also, to inoculate each leaf as uniformly as possible, so that one does not receive greater pressure than another. Each half leaf should be washed immediately after inoculation to remove any excess inoculum which might injure the leaf if allowed to dry.

The local lesion host must also be chosen with care. For tobacco mosaic virus the favourite plant in America is one or more varieties of bean (*P. vulgaris*), such as Early Golden Cluster, Pinto and others. In England, less success has been obtained with beans; Canadian Wonder, for example, usually gives no local lesions with tobacco mosaic virus. *N. glutinosa* is much used also for this virus, but its place is being taken by a variety of tobacco *Xanthi* which has had the local lesion response bred into it and so acts in the same way as *N. glutinosa*.

For tobacco necrosis viruses the bean is a suitable local lesion host, such as the varieties Canadian Wonder, Bountiful and others. It should be remembered, however, that unlike the lesions of tobacco mosaic virus, those of tobacco necrosis viruses on bean do not remain discrete but tend to coalesce if left for a few days. The lesions should therefore be counted immediately after they are fully developed.

For cucumber mosaic virus the bean is also a useful local lesion host, though some workers prefer the cowpea (*Vigna sinensis* Endl. var. Black) (Tomlinson *et al.*, 1958).

For potato virus X the best local lesion host is probably *Gomphrena globosa;* more information on these hosts can be found in Chapter IX dealing with indicator plants.

ISOLATION AND SEPARATION OF VIRUSES AND VIRUS STRAINS FROM MIXED INFECTIONS

In the early days of plant virus research it was hardly realized that some plant virus diseases might be the result of two viruses acting together in the same host. One of the first demonstrations of this phenomenon was made by Smith (1931) in his studies of the virus diseases of the potato plant.

Nowadays it is realized that composite virus diseases are extremely common, and in the case of vegetatively propagated plants they are the rule rather than the exception. This is particularly true of the woody plants such as stone-fruit trees and of herbaceous plants like the potato, strawberry, chrysanthemum, dahlias and many others.

The methods by which plant virus complexes can be resolved are very varied and they must be selected according to the properties and reactions of the viruses concerned. Put very briefly, these methods are based upon modes of transmission, insect vector relationships, the use of selective plant hosts (sometimes referred to as 'filter plants') and by taking advantage of differential properties of the viruses concerned such as resistance to ageing, thermal inactivation point, and so forth. A few examples will serve to make this clearer.

In his experiments with a potato crinkle disease, Smith (1931) inoculated by sap from the potato to tobacco, var. White Burley, and found that a severe necrotic disease was produced. However, when transmission was made to healthy plants of the same variety of tobacco from the same diseased potato plant by means of the aphid *Myzus persicae*, a different and much less severe disease was produced, consisting of a dark green banding of the veins. Since it was known that *Datura stramonium* was immune

to the vein-banding virus, the next step was to inoculate this plant directly from the same infected potato plant. Sub-inoculation from the *Datura* to tobacco produced a third disease consisting of necrotic concentric rings with a central spot. When the vein-banding viruses was added to the ringspot disease in the tobacco, or vice versa, the original necrotic disease was reproduced. The explanation is simple enough, the potato plant contained two viruses, now known as potato viruses X and Y, both of which are sap-transmissible. Therefore sap-inoculation from the potato plant gave rise in tobacco to the necrotic disease characteristic of infection in tobacco by the two viruses. Since virus X, however, is not aphid-borne, transmission by *M. persicae* produced in tobacco only the virus-banding symptoms of virus Y. Virus X was isolated by passage of the X + Y complex through *D. stramonium*, which is immune to the latter virus.

Variations of the above phenomenon are common enough; some strains of cucumber mosaic virus and henbane mosaic virus produce symptoms on the tobacco plant closely resembling those of tobacco mosaic virus. In a mixture of cucumber mosaic and tobacco mosaic virus, the first-named can be isolated by means of aphid transmission since the latter is not aphid-transmitted. To eliminate the cucumber mosaic virus it is only necessary to keep sap, extracted from the infected tobacco plant, at room temperature for a few days. This inactivates the cucumber mosaic virus and leaves the tobacco mosaic virus behind. Alternatively, heating the sap for 10 minutes at 60° C. will also eliminate the cucumber mosaic virus.

Sometimes, if a plant is found affected by an unknown virus disease, a certain amount of experimentation is necessary before the complex, if present, can be unravelled.

An example of this is the tobacco disease known as 'rosette' (Smith, 1946). Intimation that this was caused by a virus complex was given when sap-inoculation from the original rosetted plant to tobacco produced a mild mottling infection quite different from the original rosette

disease. On the other hand, unlike the potato viruses X and Y complex, aphid transmission to tobacco gave rise to the complete rosette disease.

No 'filter plant' was known by which one virus could be eliminated, so that other methods had to be tried. Finally the problem was solved by the following method: aphids *M. persicae* were colonized on a rosette tobacco plant for 48 hours and then transferred singly to young tobacco plants. Every 24 hours the individual aphids were moved to fresh tobacco seedlings; this was kept up for a period of 3 weeks. It was discovered that during the period the output of virus by the aphids varied, and sometimes one virus was transmitted and sometimes the other, but, more often, both. Thus some of the plants became infected with one of the two viruses and some with the other. The two viruses thus isolated were named tobacco mottle and tobacco vein-distorting viruses, respectively. This problem differed from that of the X and Y virus complex, in that both viruses were aphid-borne and only one was sap-transmissible.

Variations in vector-relationships can also be used in the separation of two viruses even when both viruses are aphid-borne. The simplest case of this phenomenon is given by two viruses commonly infecting *brassicae* and other cruciferous crops, the cabbage black ringspot and cauliflower mosaic viruses. The aphids, *M. persicae* and *Brevicoryne brassicae*, when colonized on cauliflower seedlings infected with these two viruses will transmit them both to healthy plants, but the aphid *M. ornatus* Laing, similarly colonized, picks out the cauliflower mosaic virus, leaving the cabbage black ringspot virus behind (Kvičala, 1945). The latter virus is easily isolated by inoculation of the complex to the tobacco plant, on which local lesions are produced and from which the virus can be obtained. The cauliflower mosaic virus does not infect the tobacco plant. *Nicotiana langsdorffii* can be substituted for the tobacco since the cabbage black ringspot produces a systemic mosaic disease in that species.

A similar procedure has recently been used in the separation of two hitherto undescribed viruses from Japan which occur almost invariably together in white clover plants. In this case only one virus is transmissible by the aphid *M. persicae*, whilst the broad bean plant (*Vicia faba*) is susceptible only to the other virus (Koshimizu & Iizuka, 1957).

Potato virus Y and cucumber mosaic virus have rather similar properties and sometimes occur together in the same plant. The aphid *M. ascalonicus* Donc. will select out the cucumber mosaic virus and leave potato virus Y behind. The latter could be separated by inoculation to the potato plant which is less susceptible to cucumber mosaic virus. The aphid *M. ascalonicus* will also select henbane mosaic virus out of a mixture of this virus and that of severe etch (Doncaster & Kassanis, 1946).

The strawberry plant is frequently infected with virus complexes, of which the component viruses are mostly aphid-borne; the aphid relationships of these viruses offer some interesting opportunities for separation experiments. For example, where one of the component viruses is non-persistent and the other persistent (see Chapter IV), separation of them can be effected by using the same aphids. Thus, the strawberry aphid, *Pentatrichopus fragaefolii*, if allowed to feed for several days on strawberry plants infected with severe crinkle disease, will pick up two viruses. One of these is the strawberry mottle virus, which is a non-persistent virus and can be eliminated from the aphids by transferring them to fresh indicator plants after 24 hours. The other, strawberry crinkle virus, is a persistent virus and has a latent period in the insect of 12–16 days (Prentice, 1949).

Separation of Virus Strains

Very few plant viruses, particularly those of the sap-transmissible mosaic type, are single entities but consist of several strains. In the case of tobacco mosaic virus there are usually one or more 'yellow' variants which show up on a mottled leaf as yellow spots of varying sizes.

It has long been known that after passage of particular plant hosts some viruses have apparently undergone a change in virulence. A classic example of this is the apparent reduction in virulence of the beet curly-top virus after passing through *Chenopodium murale* (Carsner & Stahl, 1924), and its apparent reactivation after passage of *Stellaria media* (Lackey, 1932). However, the most probable explanation is not that the virus has undergone any change but that the particular host plant favours the more rapid development of another strain of the virus which thus obscures the original virus inoculated. This is shown by two examples; Johnson (1947) inoculated the sea holly (*Eryngium aquaticum*) with virulent strains of tobacco mosaic virus and could always recover mild strains from the infected plant; similarly Matthews (1949) found that the tree tomato, *Cyphomandra betacea*, has the property of selecting out a severe strain when inoculated with potato virus X. It will be well to bear in mind, however, that apparent alterations to viruses by passage of certain hosts do occur. Bawden (1958) has shown that a particular strain of tobacco mosaic virus mutates on passage between beans (*Phaseolus vulgaris*) and tobacco, and Watson (1956) considers that potato virus C alters its insect-vector relationship after passage of Majestic potato.

A more practical method of isolating virus strains is by subinoculation from the spontaneously developing yellow spots in mottled leaves, previously mentioned, or from local lesions.

The yellow spots occur most frequently in tobacco plants infected with tobacco or cucumber mosaic viruses, and if care is taken a different virus strain from the parent can be isolated from them. The yellow spot can be cut out by means of a razor-blade and used as inoculum, but none of the surrounding green tissue should be taken at the same time. Another method is to hold the leaf of a healthy tobacco plant immediately beneath the yellow spot and prick through the spot into the leaf below with a sterile needle. By these methods yellow mottling variants of both

tobacco and cucumber mosaic viruses can be isolated. Jensen (1933, 1936, 1937) has isolated over fifty strains of tobacco mosaic virus in this way; some of his strains appear similar to what occur in nature, but others are different. Price (1934) obtained a number of variants of cucumber mosaic by cutting out similar yellow spots, and one of these yellow variants is still unchanged after twenty-five years. By its unmistakable bright yellow mottling it serves a useful purpose in cross-immunization tests with other suspected strains of cucumber mosaic virus.

There is a good deal of evidence which suggests that only one virus particle is concerned in the production of a local lesion. That being so, subinoculation of individual local lesions to plants in which the virus becomes systemic is another method for isolating virus strains. It is necessary to use a local lesion host in which the virus does not spread for obtaining the lesion inoculum. *Nicotiana glutinosa* and some varieties of bean (*Phaseolus vulgaris*) are suitable for tobacco mosaic virus, whilst some of the chenopodiaceous indicator plants such as *Gomphrene globosa* and *Chenopodium amaranticolor* can be used for other viruses (see Appendix).

A simple experiment on these lines is to dilute some crude extracted sap, from a tobacco plant infected with tobacco mosaic virus, to one part in a thousand or higher and inoculate a few large leaves of *N. glutinosa*. A number of individual lesions can then be cut out and inoculated to young tobacco plants, using one of the techniques for inoculating small quantities of virus described in Chapter VIII.

REFERENCES

BALD, J. G. (1950). In 'Viruses 1950.' Ed. M. Delbrück. *Cal. Inst. Tech.* p. 17.

BAWDEN, F. C. (1950). *Plant Viruses and Virus Diseases.* 3rd ed. Waltham Mass.: Chronica Botanica Co.

(1958). 'Reversible changes in strains of tobacco

mosaic virus from leguminous plants.' *J. gen. Microbiol.* **18,** 751–66.

CARSNER, E. and STAHL, C. F. (1924). 'The relation of *Chenopodium murale* to curly top of the sugar beet.' *Phytopathology* (abstr.), **14,** 57.

DONCASTER, J. P. and KASSANIS, B. (1946). 'The shallot aphis, *Myzus ascalonicus* Doncaster, and its behaviour as a vector of plant viruses.' *Ann. appl. Biol.* **33,** 66–68.

FRY, P. R. and TAYLOR, W. B. (1954). 'Analysis of virus local lesion experiments.' *Ann. appl. Biol.* **41,** 664–74.

HOLMES, F. O. (1929). 'Local lesions in tobacco mosaic.' *Bot. Gaz.* **87,** 39–55.

JENSEN, J. H. (1933). 'Isolation of yellow-mosaic viruses from plants infected with tobacco mosaic.' *Phytopathology*, **23,** 964–74.

(1936). 'Studies on the origin of yellow-mosaic viruses.' *Phytopathology*, **26,** 266–77.

(1937). 'Studies on representative strains of tobacco mosaic virus.' *Phytopathology*, **27,** 69–84.

JOHNSON, J. (1947). 'Virus attenuation and the separation of strains by specific hosts.' *Phytopathology*, **37,** 822–37.

KLECZKOWSKI, A. (1949). 'The transformation of local lesion counts for statistical analysis.' *Ann. appl. Biol.* **36,** 139–52.

KOSHIMIZU, Y. and IIZUKA, N. (1957). 'Origins and formation of intracellular inclusions associated with two leguminous virus diseases.' *Protoplasma*, **48,** 113–33.

KVIČALA, B. (1945). 'Selective power in virus transmission exhibited by an aphis.' *Nature, Lond.* **155,** 174–5.

LACKEY, C. F. (1932). 'Restoration of virulence of attenuated curly-top virus by passage through *Stellaria media*.' *J. agric. Res.* **44,** 755–65.

MATTHEWS, R. E. F. (1949). 'Reactions of *Cyphomandra betacea* to strains of potato virus X.' *Parasitology*, **39,** 241–4.

PRENTICE, I. W. (1949). 'Resolution of strawberry virus complexes: III. The isolation and some properties of virus 3.' *Ann. appl. Biol.* **36,** 18.

PRICE, W. C. (1934). 'Isolation and study of some yellow strains of cucumber mosaic.' *Phytopathology*, **24**, 743–61.

SAMUEL, G. and BALD, J. G. (1933). 'On the use of the primary lesions in quantitative work with two plant viruses.' *Ann. appl. Biol.* **20**, 70–99.

SMITH, KENNETH M. (1931). 'On the composite nature of certain potato virus diseases of the mosaic group.' *Proc. roy. Soc.* B, **109**, 251–67.

(1946). 'Tobacco rosette: a complex virus disease.' *Parasitology*, **37**, 21–24.

STEERE, R. L. (1955). 'Concepts and problems concerning the assay of plant viruses.' *Phytopathology*, **45**, 196–208.

TOMLINSON, J. A., SHEPHERD, R. J. and WALKER, J. C. (1958). 'Purification and serology of cucumber mosaic virus.' *Nature, Lond.* **182**, 1616.

WATSON, MARION A. (1956). 'The effect of different host plants of Potato Virus C in determining its transmission by aphids.' *Ann. appl. Biol.* **44**, 599–607.

YOUDEN, W. J. and BEALE, H. P. (1934). 'A statistical study of the local lesion method for estimating tobacco mosaic virus.' *Contr. Boyce Thompson Inst. Pl. Res.* **6**, 437–54.

CHAPTER XI

The Serology of Plant Viruses

In describing very briefly the serology of plant viruses and some of its practical applications it may be helpful first to define some of the terms used. When an animal is infected with a pathogen, whether it is a virus or a bacterium, there are produced in the blood-stream proteins which combine specifically with the virus or bacterium. This act of combination can be demonstrated *in vitro* in several ways and forms the basis of serological tests. The proteins produced in response to the stimulus of the pathogen are called *antibodies*, and they can be formed not only in response to disease agents but to many other foreign materials including normal plant proteins.

Any substance which will stimulate the production of antibodies and which will combine with them *in vitro* is called an *antigen*. A serum containing antibodies is called an *antiserum*, whilst serum from an animal which has not been injected with any antigens is called *normal*. To obtain the antiserum the rabbit is the animal generally used, though the domestic fowl and also the horse have been employed. The injections are either intraperitoneal or intraveinal and the quantity of virus (antigen) used at each injection is about 5 ml.

Since it was first shown by Purdy (Beale) in 1928 that the sap from mosaic-diseased tobacco plants contains an antigen specific for virus-containing extracts and one not present in the sap of healthy tobacco plants, much research has been carried out on the antigenicity of plant viruses. Many other plant viruses are now known to be

good antigens, especially those which are fairly stable and occur in high concentration in the plant sap. After the first discovery of Purdy Beale it was shown by Gratia (1933*a*, *b*) that plants containing different viruses contained also different specific antigens, whilst Birkeland (1934) showed that plants thought to be related contained antigens which reacted with each other's antisera.

Four types of serological reaction have been used in plant virus work.

(1) *Neutralization* of the proprties of the virus.
(2) *Complement Fixation Test*. When antigens are mixed with their specific antibodies the mixture has the property of removing the power of normal serum to haemolyse sensitized red corpuscles. It is a kind of delicate colour indicator test. Complement is a heat-labile substance present in normal blood serum.
(3) *Precipitin Reaction*. A precipitate is formed when the virus is added to its specific antiserum in saline at different dilutions and warmed in a water-bath. In precipitation the antibody is referred to as *precipitin*.
(4) *Anaphylaxis*. In this test the union between antigen and antibody is detected by reactions in animal tissues.

The technique most frequently used is the Schultz–Dale method *in vitro* (Dale, 1931). Virgin female guinea-pigs are given one injection of the antigen, and after 3 weeks the animals are killed and the horns of the uterus removed. The horns are placed in a bath of Ringer's solution at 37° C., with one end tied so that it cannot move and the other attached to a needle which can record movement on a kymograph drum. Antigen is then introduced into the Ringer solution. A positive reaction is shown by rapid contraction of the uterine horns, followed by a slow relaxation. This method was used by Chester (1936) to distinguish between antigenic contituents of healthy and virus-infected plants.

We are only concerned here with the precipitin reaction and its practical applications in plant virology, but for an excellent account of plant virus serology generally the reader is referred to a monograph by Matthews (1957).

PREPARATION OF THE VIRUSES (ANTIGENS)

Since we know that normal plant proteins are also antigens and that some of them, particularly from solanaceous plants, are toxic to animals, some preliminary clarification of the virus-containing sap is necessary.

Freezing the leaves before mincing or grinding assists in the coagulation of plant material. With some viruses having a high thermal inactivation point, heating the expressed sap to 50–60° C. for a few minutes coagulates much of the host material, which can then be removed by centrifugation. Dialysis against running tap-water for a few hours will remove much toxic material; this is important when using solanaceous plants.

With some viruses, which are stable and occur in high concentration in the infected plant, a fairly pure preparation can be obtained by means of the salt precipitation techniques described in Chapter V, or by means of ultracentrifugation. The advantages of using purified virus preparations are the absence of toxins and the possibility of giving larger injections without harmful results to the rabbit.

THE PRECIPITIN REACTION

There are four practical applications of the precipitin technique in the study of plant viruses; first the identification of a virus, secondly the detection of latent virus infections in plants, thirdly the recognition of relationships between viruses which might otherwise not be suspected, and fourthly the quantitative estimation of viruses. It is proposed to give a few examples of these applications and then to describe in some detail the practical procedures.

An early experiment of Purdy Beale (1934) was concerned with a number of strains of tobacco mosaic virus. Thus, extracts from different solanaceous plants affected with tobacco mosaic, attenuated tobacco mosaic and a yellow (aucuba) mosaic all yielded extracts giving a positive precipitin reaction with antiserum to tobacco mosaic virus. It should be realized, however, that while the precipitin test will tell whether two viruses are related to each other, the estimation of the degree of relationship is a more difficult matter.

Bawden & Pirie (1937) showed that the viruses known as Cucumber Viruses 3 and 4 have certain antigens in common with tobacco mosaic virus, long before a common host plant was known on which cross-immunity tests could be carried out. The same workers (1942) demonstrated that the virus of tobacco necrosis is in reality a number of viruses, biologically similar but serologically different. This could hardly have been demonstrated in any other way because the lesions produced on infected plants by the different viruses are so much alike.

Another important application of the precipitin technique is the detection of latent virus infections. This is especially useful in the building up of virus-free stocks of vegetatively produced crops such as potatoes, raspberries and strawberries. A latent form of potato virus X is common in potato plants and its presence cannot be detected from the appearance of the plant alone. One method of testing for the presence of potato virus X is to inoculate to one of the indicator hosts, as described in Chapter IX. This takes at least a week or 10 days, but the precipitin test can be carried out with a few precautions on the spot actually in the field, and takes only a few moments.

The same method has been used for detecting virus in symptomless carriers of Cattleya orchids (Zaitlin *et al.* 1952).

There are several ways in which the precipitin reaction can be used for estimating virus concentration. These include the precipitation end-point method, the optimal pro-

portions method and the estimation by time taken for precipitation. For a detailed account of these and other methods the reader is referred to Matthews (1957).

A short account now follows, with practical details, of the precipitin method of testing for a latent virus infection, in this case potato virus X.

As we have already pointed out, as the normal plant proteins also cause the production of antibodies, and as the saps of many solanaceous plants contain substances highly toxic to rabbits, it is advisable to use partially purified virus for preparing the antiserum. The material to be used for injection is prepared as follows:

Leaves from plants known to be infected with virus X are used as starting material. It is advisable to use potato leaves as this plant is immune to the virus of tobacco mosaic which is a frequent contaminant of tobacco and many other solanaceous plants. Antisera prepared from these latter plants will thus often react to tobacco mosaic virus as well as to potato virus X.

The leaves containing the virus are crushed in a mincer or with a pestle and mortar and about 100 ml. of sap are expressed. The sap is heated to 55° C. for about 10 minutes and the bulky green precipitate of plant material which forms is removed by centrifuging.

A volume of saturated ammonium sulphate (made by adding 760 gm. of the solid for each litre of distilled water) equal to half the volume of sap is added. This precipitates most of the virus on standing for 1 to 2 hours. The precipitate is centrifuged down and the supernatant liquid discarded. The precipitate containing most of the virus is resuspended in about 5 ml. of water (one-twentieth of the original volume of sap). The virus concentrate is then dialysed against running tap-water for several hours to remove excess of salt and any remaining plant constituents. After dialysis the solution is centrifuged to remove any debris and is then ready for injection into the rabbit.

The rabbits used for preparing the antisera should be large (about 4 lb.) and should preferably have fairly large

ears with prominent veins; the variety of rabbit known as 'half-lops' is suitable. A 1-ml. hypodermic syringe with a thin needle (about size 14) is used. The syringe is filled and air bubbles are expelled with the tip of the syringe held upright. The injection is made into the vein which runs along the upper surface of the ear, parallel to the hind edge and about $\frac{1}{8}$ to $\frac{3}{16}$ of an inch from it. It is useless to try to use the other veins even though they may appear larger.

If a rabbit is going to receive a series of injections it is preferable to give the first near the tip of the ear and each later injection successively closer to the base. The hair on the ear is smoothed down by wiping with cotton-wool dipped in alcohol, or the ear may be shaved. The tip of the needle is inserted into the vein in the direction of the base of the ear, and 1 ml. of the virus prepared as above is injected. If the tip of the needle is in the vein the liquid will flow in smoothly. The needle is then withdrawn gently, slight pressure being maintained over the point of entry with the thumb of the left hand.

About 2 weeks after injection the rabbit is bled from the other ear. For this the ear is prepared by rubbing a very small amount of xylene or benzene on to its surface with a small wad of cotton-wool. This is slightly irritant, causing the veins to fill with blood, and allows the bleeding to be done quickly and with a minimum of discomfort to the rabbit. A small cut is then made in the marginal vein near the base of the ear, using a small very sharp scalpel. The type having interchangeable blades (Swann-Morton, No. 3, Blades No. 11) is very suitable.

After sufficient blood has been taken the flow can be stopped by applying slight pressure, and the cut sealed by a small quantity of collodion dissolved in alcohol-ether.

The cuts for later bleedings are made successively nearer the tip of the ear. The blood is collected in a tube and left for some hours to clot. The serum is poured off and centrifuged to remove any remaining blood-cells. About 40 to 50 ml. of blood can be taken on three successive days followed by a further single bleeding at the end of a week.

This gives about 100 ml. of serum from one injection. The same rabbit after a rest period of a few weeks can then be used again.

Antiserum prepared in this way is not sterile and will deteriorate unless stored under conditions which prevent bacterial growth. This may be prevented either by keeping the material frozen or by the addition of a few drops of chloroform. In either case it is advisable to keep the serum as cold as possible.

The above procedure of a single intravenous injection of semi-purified virus followed by bleeding from the ear has a twofold advantage in that it is the most efficient way of producing an anti-serum and causes the animals a minimum of discomfort. The use of crude plant sap injected intraperitoneally and the heart-puncture technique for obtaining blood are not to be recommended from the above points of view.

TESTING SINGLE PLANT

About 5 to 10ml. of the sap expressed from the leaf sample to be tested is heated at 55° C. for 10–15 minutes. The bulky green precipitate is centrifuged off and the brown supernatant liquid is used for the test, at a dilution of 1 in 20. All the dilutions in these tests are made with 0·9 per cent. sodium chloride solution. For carrying out the tests small glass tubes having an internal bore of about $\frac{5}{16}$ in. are used. These are set up in racks in a water-bath with the temperature controlled at 50° C. For one full-time operator it is convenient to have rack accommodation for 50 to 100 tubes. Most suitable is a bath with windows in front and rear—with illumination from behind. The tubes can then be observed without removal from the bath.

In each of two tubes 0·5 to 1·0 ml. of the clarified test sap is placed. To one is added an equal volume of the virus X specific antiserum at a suitable dilution (previously determined, as described in a subsequent paragraph). To the other is added an equal volume of a similar dilution of

normal serum, i.e. serum obtained from a rabbit which has had no injection with the virus under test. This control mixture is necessary, as occasionally spontaneous precipitation of the sap occurs.

When the antiserum has been added the contents of each tube are mixed by brief shaking, and the lower half of the tube immersed in the water. This causes a continuous circulation of the contents of the tubes. If virus X is present in the test sample a specific precipitate often appears within a few minutes. It may be recognized as small, fluffy, almost transparent particles circulating in the tube. These particles gradually clump together and settle to the bottom of the tube as a whitish flocculent precipitate. The tubes should be examined for a precipate after a few minutes, again after about half an hour, and after about one hour. Specific precipitates rarely form (with the dilutions used) after one hour. After some hours in the bath, non-specific precipitates of plant material from the sap will probably form. These precipitates are usually more compact and darkly coloured than the specific precipitates.

If the heat clarification of the sap has been carried out efficiently, non-specific precipitates should not form for some time, if at all. The appearance of a specific precipitate is a definite demonstration that the plant from which the sap was obtained is infected with the virus. The absence of a specific precipitate, although not proof, is a reasonable indication that the plant is free of virus X. Starting from uncrushed leaf samples, it is possible for one operator to do about 100 tests in a working day.

To determine a suitable dilution of a newly prepared antiserum for use in the tests the following preliminary test is carried out. A series of two-fold dilutions of the antiserum is set up in small tubes, and an equal volume of heat-clarified X infected sap at a dilution of 1 in 20 is added to each dilution of antiserum. The set of tubes is placed in the water-bath at 50° C. and the highest dilution of antiserum to form a specific precipitate within one hour is noted. A strength of antiserum two to four times as concentrated as

this minimal concentration is used for carrying out the tests.

SEROLOGICAL GROUP TESTING

Just as the method of inoculating from single potato plants can be modified for testing large numbers by a grouping of the plants to be tested, so serological group testing can be carried out. The leaf samples from 10 or 20 plants are combined and the expressed sap from these is tested as a single sample. The only modification of the technique described above for single plants is that the heat-clarified sap is used undiluted instead of being diluted to 1 in 20. If only one plant in the group of 10 or 20 is infected the dilution in the final test sample will be 1 in 10 or 1 in 20. This concentration of virus is quite sufficient to give a good specific precipitate.

A point of some importance in the sampling is that the sample from each plant of a group should be about the same size. If the sizes of samples from different plants of a group varied widely, then it would be possible for a single infected plant to be 'diluted' a good deal more than the 1 in 20.

GENERAL CONSIDERATIONS

(1) One rabbit from one series of bleeding can give about 100 ml. of antiserum. If 0·5 ml. quantities of a dilution of 1 in 50 of the antiserum are used, this amount of antiserum is sufficient for 10,000 single plant tests. If groups of 10 plants are to be tested, 100 ml. of antiserum would be sufficient to test 100,000 plants.

(2) There are certain strains of virus X, not commonly found in the field, which give practically no symptoms on the usual test plants and could easily be missed in inoculation tests. The serological test can pick up this type of strain just as easily as any other.

(3) The facilities required for serological testing are not

as extensive as for plant-inoculation methods, the chief requirements being a suitable water bath to run at 50° C., a small power-driven centrifuge to take eight to twelve 15-ml. tubes and a supply of small tubes and pipettes.

(4) The carrying out of serological testing is not limited by the seasons as the growing of test plants may be.

(5) The result of the test is known within an hour or two compared with 2 to 3 weeks for the inoculation methods.

THE COLLECTION OF SAMPLES

The following considerations apply, whatever method of testing for the virus is used. In order to find out whether a potato plant is completely virus-free it is necessary to test leaflets taken from each shoot. It is recommended that this should be done if the plants are to be used as the nucleus from which virus-free stocks are to be derived.

On the other hand, if the testing is to be carried out on stocks in large-scale production the probability of only one shoot on a plant being infected is small, and it is unlikely that sampling from one stem of each plant will give a result different from sampling from each shoot of every plant sampled.

If single-plant tests are being made, then one whole leaf from each plant could be taken. If group testing is used, then a single leaflet would be sufficient. With serological testing sufficient material must be taken to give about 5 to 10 ml. of sap for clarification from each sample or group. With group testing it is advisable to take about the same sized sample from each plant. The youngest fully expanded leaves on a stem are probably the most suitable for sampling.

It is obvious that leaflets cannot be taken from each plant even when only a few acres of potatoes are grown. Therefore a sample must be taken, as representative as possible of the crop as a whole. A suitable way is to take plants at random in a diagonal line across the field.

As a sample of the crop has to be taken it may not be

exactly representative of the crop as a whole. Deductions made from tests on such a sample are never completely reliable, but their reliability increases as the size of the sample increases. In Table I are tabulated the numbers of plants which have to be tested and found to be free from virus in order to ensure with different degrees of reliability a certain standard of health in a large crop. The numbers of plants under column A will give a reliable result 90 times out of 100, while those in columns B and C are reliable in 95 and 99 cases out of 100, respectively.

TABLE I

The numbers of plants which have to be tested and found to be virus-free in order to ensure a certain standard of health in a large crop

Standard of health to be tested	Size of sample (i.e. number of plants) that must be tested		
	A	B	C
Less than 1% virus infection	230	300	460
Less than 0·1% virus infection	2,300	3,000	4,600
Less than 0·01% virus infection	23,000	30,000	46,000

For general use column B will be adequate. For instance, if one plant in every 1,000 plants is infected a random sample of 3,000 plants will include at least *one* virus-infected plant 95 times out of 100 (Markham, Matthews & Smith, 1948).

Govier (1958) has described a slightly different technique for preparing antisera to potato virus X and one which does not necessitate much preliminary virus purification. Heat-clarified sap from tobacco plants infected with virus X is emulsified in an equal volume of Difco Bacto-adjuvant Complete (Freund) and 1 ml. of the emulsion is injected intramuscularly into each of the hind legs of the

rabbit. Bleedings are taken at intervals after the injection and the antiserum titre reaches its maximum after about 4 weeks. The antiserum titre is maintained at a near maximum level over a long period following intramuscular injection, and a large volume of high titre antiserum can be obtained without resorting to further injections.

Some experiments in France (Payen & Madec, 1957) will show how the presence of an apparently new virus in potato plants can be demonstrated serologically. An antiserum from Ratte potatoes reacted positively with sap from the plants from which it had been prepared, and negatively with sap from healthy Bintje potato plants. The mosaic disease in Ratte plants is accompanied by a slight, soft rolling of the leaflets near the top of the plant. Tests demonstrated that the antibodies of the serum were not due to special proteins in the Ratte variety and were not specific for virus A; it was therefore suspected that the infected Ratte plants contained a virus causing the mosaic and soft leaf-roll symptoms. Other tests of 53 potato varieties affected by mosaic showed that 6 were completely and 16 partially infected. Within the variety Fin de Siècle, 16 plants reacted positively towards the Ratte antiserum but negatively to antisera for potato viruses X, Y and S.

Another experiment illustrates how relationships between certain viruses affecting the genus *Brassica* can be demonstrated serologically. An attempt was made to prepare antisera for seven virus isolates, cabbage black ring virus, cabbage virus A, cabbage black ringspot virus, horse radish mosaic virus, cabbage virus B, cauliflower mosaic virus and Chinese cabbage mosaic virus.

For cabbage black ring virus, cabbage viruses A and B, cauliflower mosaic virus and Chinese cabbage mosaic virus, the expressed infective sap from frozen tissue was centrifuged for 30 minutes at 3,000 r.p.m. and used for injection of rabbits. Successive intravenous injections of 0·5, 1, 1·5, 2 and 2·5 ml. of clarified sap were given in each case, at 2-day intervals, followed by bleeding 10 days after the last injection. Two or three rabbits were injected with sap from

each source of virus. For cabbage virus A and cabbage black ringspot virus, sap from systemically infected plants of *Nicotiana glutinosa* L. was used for injection; for cabbage virus B, cauliflower mosaic virus and Chinese cabbage mosaic virus, it was necessary to use sap from recently infected young cabbage plants. The cabbage virus A and cabbage black ring virus antisera reacted with healthy *N. glutinosa* sap but not with healthy cabbage sap. The antisera prepared by the injection of infected cabbage sap, however, did not react visibly with healthy cabbage sap. Antisera for horseradish mosaic virus and cabbage black ringspot virus were prepared by intravenous injection of 2 ml. of infective heat-clarified sap from *N. glutinosa*. For testing the antisera, all isolates were taken from young, recently infected cabbage plants. Infective sap prepared by centrifugation alone gave fair clarification; but better clarification was obtained by heating to 53° C. for 4 minutes and then centrifuging. There was no detectable loss of those viruses that produced reactive antisera.

Precipitation in tubes in a water-bath held at 30° C. was found to be most satisfactory. With this procedure no non-specific precipitation occurred for several hours even with infective sap that had been clarified by centrifugation only. In all tests appropriate controls were set up with healthy sap as well as with normal serum. The reactive sera gave titres up to 1 : 16, while the highest virus end-point dilution found was 1 : 32 for the cabbage black ringspot virus. All precipitations were carried out by the addition of 0·5 ml. of undiluted sap to 0·5 ml. of undiluted serum.

No positive reactions with any antisera were obtained with cabbage virus B, Chinese cabbage mosaic virus or cauliflower mosaic virus. However, the positive tests obtained with the other viruses show that cabbage black ringspot virus, cabbage black ring virus, cabbage virus A and horseradish mosaic virus are all serologically related. The specific precipitates obtained with this group of viruses were of the rapidly forming open flocculent 'H' type commonly produced by rod-shaped viruses such as those

of tobacco mosaic and potato virus X (see Chapter VII) (Larson, Matthews & Walker, 1950).

It may perhaps be pointed out that the above aphid-transmitted viruses affecting cruciferous crops fall naturally into two groups by reason of their host range. Those viruses, of which cabbage black ringspot virus is the type, affect solanaceous plants and also many other families and produce on *N. tabacum* local lesions of a closely similar type without further systemic spread. The cauliflower mosaic group, on the other hand, will not infect *N. tabacum* or any other solanaceous plant.

SOME RAPID SEROLOGICAL TECHNIQUES

These methods are intended for field diagnosis and are not suitable for accurate work.

Munro (1954), basing his procedure on methods described by Chester (1937) and by van Slogteren (1944), devised a simple slide agglutination technique. A drop of sap from a potato leaflet is placed at each end of an ordinary microscope slide; to one is added a drop of virus X antiserum and a drop of normal serum to the other. The drops are then stirred with opposite ends of a wooden toothpick which is then discarded. Agglutination in sap from an infected plant usually occurs within about 10 seconds of stirring. The stirred drops can be examined almost immediately, and if the slide is held at the junction of a light source, such as a mirror, and a paper the agglutination can be clearly seen.

Stapp & Bercks (1948) used dried antisera instead of drops. They impregnated thin white paper with antisera to potato virus X and dried it in a desiccator over calcium chloride. Wafers 4 mm. square are cut from the sheets and from sheets treated similarly with normal serum. A wafer of each type is put at opposite ends of the slide and a drop of 0·9 per cent. saline is added, followed by a drop of the sap to be tested. The latter was partially purified by low-speed centrifugation. The slide must then be incubated at

23° C. for about 20 minutes and examined in a microscope by dark ground illumination.

A simpler method has been described by Świeżyński (1950); he substituted a porcelain spot test plate with twelve depressions for the glass slide and mixed a drop of crude sap with a drop of dilute antiserum. The plate is shaken to mix the drops and after 3–5 minutes the agglutination can be observed. A green aggregate appearing on the edge of the mixture indicates the presence of virus. When no virus is present, no aggregate is visible and the mixture remains transparent.

REFERENCES

BAWDEN, F. C. and PIRIE, N. W. (1937). 'The relationships between liquid crystalline preparations of cucumber viruses 3 and 4 and strains of tobacco mosaic virus.' *Brit. J. exp. Path.* **18**, 275.

(1942). 'A preliminary description of some of the viruses causing tobacco necrosis.' *Brit. J. exp. Path.* **23**, 314.

BEALE, PURDY H. (1928). 'Immunologic reactions with tobacco mosaic virus.' *Proc. Soc. exp. Biol. N.Y.* **25**, 702.

(1934). 'The serum reactions as an aid in the study of filterable viruses of plants.' *Contr. Boyce Thompson Inst. Pl. Res.* **6**, 407–35.

BIRKELAND, J. M. (1934). 'Serological studies of plant viruses.' *Bot. Gaz.* **95**, 419.

CHESTER, K. S. (1936). 'Serological tests with Stanley's crystalline tobacco mosaic virus protein.' *Phytopathology*, **26**, 715.

(1937). 'A simple and rapid method for identifying plant viruses in the field.' *Phytopathology*, **27**, 722.

DALE, H. H. (1931). 'The use of isolated plain muscle in testing for anaphylaxis.' *A System of Bacteriology in Relation to Medicine*, **9**, 229. Med. Res. Council.

GOVIER, D. A. (1958). 'The preparation of antisera to

potato virus X.' *Scot. Plant Breed. Sta. Rep. 1958*, 77–81.

GRATIA, A. (1933*a*). 'Pluralité antigenique et identification serologique des virus de plantes.' *C.R. Soc. Biol. Paris*, **114,** 923.

(1933*b*). 'Qualité antigenique des virus de plantes et des bacteriophages.' *C.R. Soc. Biol. Paris*, **114,** 1382.

LARSON, R. H., MATTHEWS, R. E. F. and WALKER, J. C. (1950). 'Relationships between certain viruses affecting the genus *Brassica*.' *Phytopathology*, **40,** 955–62.

MARKHAM, R., MATTHEWS, R. E. F. and SMITH, K. M. (1948). 'Testing potato stocks for virus X.' *Farming*, February, pp. 41–46.

MATTHEWS, R. E. F. (1957). *Plant Virus Serology*, Camb. Univ. Press.

MUNRO, J. (1954). 'Maintenance of virus X-free potatoes.' *Amer. Potato J.* **31,** 73.

PAYEN, O. and MADEC, P. (1957). 'Sur la présence en France d'un nouveau virus de la pomme de terre.' *C.R. Acad. Agric. France*, **43,** 265–7.

STAPP, C. and BERCKS, R. (1948). 'Über weiters Antrocknungsversuche mit seren gegen Kartoffelviren.' *Phytopath. Z* **15,** 47.

ŠWIEŻYŇSKI, K. (1950). 'A simplified serological test for the determination of virus X in potato plants.' *Acta Societatis Botanicorum Poloniae*, **20,** 513–21.

VAN SLOGTEREN, E. (1944). 'De Herkenning van Virus-Ziekten der aardappelen langs serologische Weg (Netherlands).' *Lab. Bloembollen-oenderzoek, Lisse*, No. 76.

ZAITLIN, M. A., SCHECHTMAN, A. M., BALD, J. G. and WILDMAN, S. G. (1952). 'Rapid serological detection of virus in Cattleya orchids.' *Phytopathology* (abstr.), **42,** 478.

CHAPTER XII

The Purification of Plant Viruses

When investigating a possibly undescribed sap-transmissible virus it is desirable to have some information on certain elementary properties of the virus before commencing more comprehensive studies on its purification. What is wanted is some information on its stability and concentration in the plant because attempts at isolation are likely to be much more difficult if the virus in question is extremely labile or occurs in very low concentration. The three elementary tests usually carried out are to determine the ageing, or longevity *in vitro*, of the virus in extracted sap, the thermal inactivation point and the dilution end-point. It should be realized that the results of these tests are likely to be approximate only, since much may depend on the source of the inoculum, the availability of a good indicator host and other factors. In making the tests there are several conventions which should be followed, and there must be a preliminary clarification of the virus-containing sap because the presence of whole cells would obviously vitiate the results. The sap is expressed from the infected plant usually with a pestle and mortar and filtered through a piece of fine muslin or cheese cloth; it should then be clarified by low-speed centrifugation.

LONGEVITY *IN VITRO*

The clarified sap is placed in a small conical flask and kept in the laboratory at room temperature. Inoculations are made at intervals to an appropriate test plant. At first the test may be made every 24 hours, but this interval can, of

course, be increased if the infectivity seems likely to last for weeks or longer.

DILUTION END-POINT

Using clarified sap a number of dilutions are made, a fresh pipette being employed for each dilution. Beginning with a dilution of 1:10 the series is usually carried to 1:1,000,000, but infections are likely to cease considerably before that. If they cease at 1:100, purification will probably be a difficult problem.

THERMAL INACTIVATION POINT

The conventions here are to use a thin-walled test-tube to hold the clarified sap and to give an exposure of 10 minutes to a range of temperatures. The bulb of the thermometer is immersed in the sap, the test-tube is placed in a water-bath and held at the required temperature for 10 minutes. The test-tube is then cooled under the tap and inoculations are made to the test plants. The usual range of temperatures tested is 45° to 80° C. in 5-degree steps. It will be found that plant viruses vary greatly in their thermal inactivation points, but the majority are between 50° and 60° C.

PURIFICATION METHODS

The problems involved in separating a virus from the plant cell contents are numerous and are only partially solved. Only a small percentage of the large number of sap transmissible viruses known have been successfully purified. There are several points of practical importance to be considered before the purification of a virus can be accomplished. First, it is important that the virus should be present in sufficient concentration to make the attempt worth while; some information on this point can be obtained with the dilution end-point test described above. Markham (1959) considers that the minimum quantity of virus necessary is of the order of 5 to 10 mg. of dry virus per kilogram of fresh leaf material, but for consistent successful purification a larger quantity than this is desirable. Another important point is the plant used as a

source of virus; many factors are concerned here. As a rule, young plants, showing recent systemic symptoms, have a higher virus content than old plants long infected and contain less pigment. This is not an invariable rule, however, since turnip yellow mosaic virus occurs in higher concentration in old hard long-infected plants of Chinese cabbage or turnip than in young sappy plants recently infected.

Some plants are unsuitable for use as source plants; those that contain large quantities of gums, latex or tarry materials, for example. Certain plants, such as New Zealand spinach (*Tetragonia expansa*), which contain inhibitors, are also to be avoided, as are strawberry plants because of their associated tannins.

There are two main methods of purification of plant viruses; by chemical precipitation methods and by sedimenting the virus on the high-speed centrifuge. To extract the virus the diseased plants should be minced; a domestic meat grinder with a worm which compresses the material before it reaches the cutters is most satisfactory. The extraction of the virus is helped if the leaves are frozen at about -10° C.; they should be stored in polythene bags and not allowed to dry. The leaf tissue is then thawed and minced; the wet pulp is pressed by hand through muslin and the sap collected. The pulp residue is then put in a hand or hydraulic press and the remainder of the sap collected.

The next step is the clarification of the sap, and this cannot be done by centrifugation alone but some chemical or physical treatment is also needed. Markham (1959) describes the two methods of sap clarification which are most satisfactory. One is the addition of 300 ml. of 90 per cent. ethanol to each litre of strained sap with vigorous stirring. This procedure causes the immediate formation of a coagulum which may be centrifuged off at low speed, leaving a golden, slightly cloudy fluid which contains the virus. There are two possible drawbacks to this method; one is that there may be viruses which cannot tolerate this

level of ethanol; the other is that the virus may be precipitated by weak ethanol at the pH of the sap.

The other method of clarification is to heat the sap to 55° C., when a coagulum forms and can be removed by centrifuging. Many viruses will tolerate 55° C. for a short time, but some will not, and there are some saps, such as those from the crucifers, which will not clarify readily by heat.

The two precipitating agents most commonly used are alcohol and ammonium sulphate. The alcohol can be used for precipitating either the virus or, as we have already seen, the extraneous plant proteins according to the particular virus being used or the strength of the alcohol. In order to make the various procedures clearer to the reader, the purification of a few common plant viruses will be described in detail.

The following description of the purification of turnip yellow mosaic virus (Markham & K. M. Smith, 1949) is taken from Markham (1959). The best source plants are Chinese cabbage or turnips, the former grow better under glass, and the virus content is higher in old pot-bound plants than in those which are young and sappy. The plants should be harvested about 2 months after infection and then ground and the sap expressed.

The sap is clarified by the addition of 300 ml. of 90 per cent. ethanol to each litre of sap, the flocculent precipitate of plant proteins spun off, and the supernatant liquid, which is yellow and slightly opalescent, has a half-volume of saturated ammonium sulphate in water added. Crystallization of the virus begins in a few minutes, and is complete in 4 or 5 hours. At the same time, strongly birefringent crystals, possibly of calcium sulphate, are also found, but these are insoluble in water, so that the pellet of crystals obtained on centrifuging may be extracted with water and reprecipitated as crystals. Under normal conditions three or four recrystallizations of the virus from ammonium sulphate suffice for the purification. Crystallization follows smoothly after enough salt has been added

to make the solution slightly cloudy, and it is virtually impossible to prepare the virus in an amorphous state. The crystals are octahedra. The reader should realize, however, that the turnip yellow mosaic virus is unusually easy to purify.

Potato virus X is a rod-shaped virus and was first purified by Bawden & Pirie (1938), whose method is given in some detail.

Plants of tobacco and *Nicotiana glutinosa* are preferable and should be inoculated when small; they are best harvested about four weeks after inoculation. The leaves are minced and the sap expressed through muslin, heated to 66° C. and then rapidly cooled. This causes a green flocculent coagulum which can be removed by centrifuging at 3,000 r.p.m. for a few minutes. The heating must be done carefully with continuous stirring to prevent local overheating since the virus is rapidly inactivated at temperatures near 66° C.

The brown opalescent fluid can now be brought to quarter saturation with ammonium sulphate (185 gm. per litre) or brought to pH 4·5 by the addition of sulphuric acid. These treatments produce a brown precipitate containing all the virus. After centrifuging, the precipitate from 1 litre of sap is suspended in 100 ml. of water, neutralized with dilute NaOH and centrifuged to remove insoluble materials. If the resulting solution is now shaken between crossed Nicol prisms it should show the phenomenon of double refraction. The virus is again precipitated by the addition of from one-third to one-half of a volume of saturated ammonium sulphate solution, centrifuged and the coloured supernatant fluid discarded. The precipitate is suspended in water and the precipitation with ammonium sulphate repeated until the supernatant fluid is no longer brown. About six repetitions of this treatment are necessary and a few drops of dilute NaOH solution must be added each time to keep the solution neutral. When no further colour can be removed by precipitation with ammonium sulphate, the precipitate is again dissolved at

pH 7 and centrifuged until free from insoluble material. The turbid, brown supernatant fluid is adjusted to about pH 4·5 with acetic acid, and the flocculent precipitate produced is centrifuged down. Generally the whole or the greater part of the virus precipitates at this pH. The acid precipitate is freed from ammonium sulphate by repeatedly suspending in water, centrifuging and discarding the supernatant fluid. The well-washed, virus-containing precipitate at pH 4·5 is dissolved by the addition of sufficient N/20 NaOH to raise the pH to 7 and the solution is centrifuged till clear.

It is possible to clean up impure preparations of tobacco mosaic virus by incubating them with trypsin because this virus is not inactivated by trypsin. Now although potato virus X *is* acted on by trypsin it is nevertheless possible to purify coloured preparations of this virus with trypsin if the sacrifice of about one-third of the virus is not objected to. One incubation with trypsin is usually sufficient to give colourless preparations, but sometimes it is necessary to repeat the treatment. The ammonium sulphate is removed by precipitating the virus at pH 4·5 and washing with water. The virus is dissolved in sufficient N/10 NaOH to bring the pH to 7. Before the last centrifugation at pH 4·5 the preparation should be frozen and thawed, otherwise the neutral solution will be too dilute to give a liquid-crystalline layer. With experience it is possible to obtain colourless solutions which, if more concentrated than about 3 per cent., will separate on standing into two layers. The lower layer is liquid crystalline, has the greater solid content and is clear, while the upper layer is turbid and shows double refraction.

Some viruses cannot be purified by chemical precipitation methods either because they are too unstable or occur in too small quantities within the plant. In such cases high-speed centrifugation is employed and was used by Stanley (1939) to isolate the virus of tobacco ringspot. Steere (1956) re-examined the purification of this virus, using Caserta squash (*Cucurbita pepo*) and Petunia as propaga-

tion hosts instead of the more usual tobacco plant. Caserta squash was used for most of the work because of the rapidity with which a supply of virus could be obtained from the planting of the seed. Steere found that the best yields were obtained when plants were shaded with one layer of newspaper for 2 days following inoculation. This appeared to increase the spread of virus within the inoculated cotyledons and into the crown of the plant. No special preharvest treatment was necessary, and no buffer had to be used during grinding and juice extraction. Steere introduced a new method for clarification of the sap; he used a mixture of equal volumes of butanol and chloroform at the rate of two volumes of the mixture to one volume of juice. The aqueous phase thus obtained was subjected to three successive sedimentations at high speed out of 0·01M phosphate buffer of pH 7. The centrifuge pellets were resuspended in water before the phosphate was added because the pellets do not resuspend readily in the buffer.

Purification of the aphid-transmitted viruses is rather difficult, mainly because of instability and low concentration of this type of virus. Bawden & Pirie (1939) carried out experiments on potato virus Y and henbane mosaic virus, using ammonium sulphate precipitation and high-speed centrifugation. They isolated a rod-shaped virus particle which showed double refraction.

As an example of the purification of an aphid-transmitted virus some recent work on the purification of cucumber mosaic virus is relevant here and the following account is quoted from Tomlinson *et al.* (1958). A strain of the virus was cultured in leaves of tobacco plants (*Nicotiana tabacum* L. var. Havana 423), mechanically inoculated with sap of corollas from infected cucumber plants. The inoculated plants were grown at 24–30° C. in a glasshouse from May until October. The inoculated leaves were harvested, the mid-veins removed and the tissue homogenized at 3° C. in 0·5M potassium phosphate buffer (pH 7·5) containing 0·1 per cent. thioglycolic acid (1·25 ml. of buffer for each 1·0 gm. of leaf tissue). The homogenate was squeezed

through a glasswool pad and *n*-butanol was added dropwise and constantly stirred for a further 30 minutes, during which time the chloroplasts, &c., were precipitated. The precipitate was separated by centrifuging at 5,000 r.p.m. for 10 minutes and the clear, amber-coloured supernatant was filtered through glass-wool. The virus was sedimented by centrifuging the filtrate at 30,000 r.p.m. After ultracentrifugation the supernatant was discarded, the centrifuge tubes drained and the pellet in each tube dispersed in 0·5 ml. of 0·05M potassium phosphate buffer at pH 7·5. Re-suspension of the virus was continued by mechanical shaking for 2 hours, after which the suspension was clarified by centrifugation at 5,000 r.p.m. for 10 minutes. The faintly opalescent supernatant containing the virus was carefully withdrawn.

For more detailed accounts of the separation and purification of plant viruses the reader is referred to Markham (1959) and Russell Steere (1959).

REFERENCES

BAWDEN, F. C. and PIRIE, N. W. (1938). 'Liquid crystalline preparation of potato virus X.' *Brit. J. exp. Path.* **19,** 66–82.

(1939). 'The purification of insect-transmitted plant viruses.' *Brit. J. exp. Path.* **20,** 322–9.

MARKHAM, R. (1959). 'The biochemistry of plant viruses.' *The Viruses.* Vol. 2. *Plant and Bacterial Viruses.* Eds. F. M. Burnet and W. M. Stanley. New York: Academic Press.

MARKHAM, R. and SMITH, K. M. (1949). 'Studies on the virus of turnip yellow mosaic.' *Parasitology*, **39,** 330–42.

STANLEY, W. M. (1939). 'The isolation and properties of tobacco ringspot virus.' *J. biol. Chem.* **129,** 405–28.

STEERE, R. L. (1956). 'Purification and properties of tobacco ringspot virus.' *Phytopathology*, **46,** 60–69.

(1959). The purification of plant viruses.' *Advances in Virus Research* **6,** 1–73.

TOMLINSON, J. A., SHEPHERD, R. J. and WALKER, J. C. (1959). 'Purification, properties and serology of cucumber mosaic virus.' *Phytopathology*, **49,** 293–9.

CHAPTER XIII

The Control of Plant Virus Diseases

There are various methods of approach to the question of the control of plant virus diseases; or to put it another way, to prevent the spread of plant viruses. They are not of course all applicable in the same way to the various diseases. These methods can be classified roughly under six headings, and the application of each of them to specific virus diseases will be briefly discussed:

(*a*) Elimination of the sources of virus infection.
(*b*) Avoiding the insect vectors.
(*c*) Direct attack on the insect vectors.
(*d*) Breeding resistant varieties of crops.
(*e*) Cure of virus-infected plants.
(*f*) Special methods of propagation.

To these may perhaps be added the 'vaccination' of a plant with an avirulent or masked strain of a virus which thereby sometimes immunizes the plant against a more severe strain of the same virus but not of course against a different virus. This method is at present largely academic.

Since the last edition of this book was published considerable advances have been made in the cure of virus-infected plants, and also some success has been achieved in the direct attack on the insect vectors by means of the new insecticides.

ELIMINATION OF THE SOURCES OF VIRUS INFECTION

Wild Host Plants

Many weeds are potential sources of virus infection for

cultivated crops, and the perennial and biennial weeds are, of course, of greater importance than annuals. The precise importance of weeds generally as a source of virus infection is difficult to assess, but there are some well-authenticated instances. Thus in Great Britain the biennial wild beet, *Beta maritima* L., is frequently infected with the virus of beet yellows and beet mosaic. The dandelion (*Taraxacum officinale* Web.) is susceptible to a virus causing a bright yellow mottle, and occasionally this virus is carried by aphids to lettuce in which it causes a severe disease.

In Africa the virus of tomato spotted wilt is frequently carried to tobacco and tomato from infected weeds by the thrips, *Frankliniella schultzei* Trybom, which settle at random on the plants but do not breed there (Van der Plank & Anderssen, 1944). The same virus is transmitted to pineapples in Hawaii by *Thrips tabaci* from the weed *Emilia sanchifolia* DC.

Weeds also harbour, frequently without symptoms, one or more soil-borne viruses which later infect cultivated crops growing in the same soil. Cadman (1956) has shown that a ringspot virus from weeds will infect raspberries, causing a leaf-curl disease.

Cultivated Crops

Viruses which have a fairly wide host range can be brought to one crop from another cultivated crop. For example, clover is the host of several viruses which affect peas and beans; moreover, the chief aphid vector, *Macrosiphum pisi*, over-winters on clover. It is unwise therefore to grow perennial leguminous crops in close proximity to susceptible annual crops. The virus of cucumber mosaic is one which lurks in many perennial garden plants, pentstemon, lupins, *Buddleia* and even in privet hedges. There is thus always a source of virus ready to attack vegetable marrows or outdoor cucumber.

Remnants of the previous year's crop are frequently important sources of virus. 'Volunteer' potatoes and

sugarbeet, which are usually infected with one or more viruses, are good examples. Mangold clamps, too, are liable to be reservoirs of the beet yellows virus.

At one time it was the practice to grow the steckling, or seed plant, sugar-beets alongside or close to the root crops. The effect of this was that the seed plants became infected with virus yellows in the autumn; the aphid vectors wintered on the plants and the following spring infected the new season's beet crop over very large areas. Now some attempt is being made to grow the seed-beets in isolation from the root crops and so to keep the latter free of virus.

With vegetatively propagated crops such as strawberries, raspberries, potatoes and bulb plants it is supremely important to start with a virus-free crop. The foundation of good crops of potatoes is the use of good quality 'seed', since it is essential to start the crop with as little virus in it as possible; otherwise there will be a source of virus ready to hand within the crop. Having obtained a stock of clean 'seed' it should be grown as far as possible from second-rate potatoes. All the advantages of virus-free 'seed' will be lost if the crop is grown alongside home-saved 'seed'.

Another important point is careful attention to roguing out any obviously virus-diseased plants and also any ground-keepers which are usually a prolific source of virus infection. Roguing should be done as early as possible while the plants are still small; there are several reasons for this. There will have been less time for the virus to have spread from the infected plants, no tubers will have been formed and small plants are easier to dispose of.

AVOIDING THE INSECT VECTORS

The growing of seed potatoes in particular areas of Scotland is a practical illustration on a large scale of the control of potato virus diseases by avoiding the insect vectors. The climate of the Scotch seed-growing districts is too cool and moist for the aphid *Myzus persicae*, the chief vector which does not thrive if the temperature is lower than about

65° F. and if relative humidity is more than 75 per cent. Similarly in some tropical countries it is possible to raise seed potatoes in areas where the temperature is too high for the aphid to exist.

Investigation of the life history of the chief potato aphid, *M. persicae*, shows that this insect can be partially avoided even in England. There are three possible ways in which the aphid can pass the winter; as an egg on the peach tree, in glasshouses, and, in mild winters, out of doors on brassica crops, particularly brussels sprouts. Whenever practicable, therefore, potato crops should be grown in areas where *M. persicae* cannot find these facilities for over-wintering.

Sometimes it is possible to avoid a bad infestation of an aphid vector by early sowing. Thus, early sowing of the sugar-beet commercial crop is recommended because it avoids the infestation by *M. persicae* of the very young beet-plants and allows them to be more advanced before the appearance of the aphid.

The condition of the plant itself may play some part in determining the degree of infestation by aphids, and Kennedy (1958) has shown that some aphids prefer the old leaves to the younger for colonization. Even the colour of the plant may play a part, since three times as many aphids alighted on green or yellow lettuce plants as on brown ones, and the brown variety is less frequently infected with lettuce mosaic than the green or yellow (Müller, 1956).

A more positive method of avoiding the insect vectors of viruses is to ward them off the crop by means of screens. This has been done in the U.S.A. against the leafhopper which spreads the virus causing 'aster yellows'. Two types of shield have been tested: one consisted of cloth-covered sidewalls or fences without tops, but these were found commercially unsatisfactory; in the second type, cloth-covered cages or houses were employed. The tops and sides of the enclosures were completely covered with cloth not coarser than 22 by 22 threads per inch. It might be worth while experimenting with some such device to protect

young cauliflower or other brassica seedlings since it is in the seedbed before being set out in the field that much infection with aphid-borne viruses takes place. It is sometimes worth while to protect a growing crop with a living screen made of some rapidly growing plant like the Jerusalem artichoke; this helps to prevent the influx of winged aphids. Similarly mosaic can be warded off cauliflower seedlings by interplanting rows of cereal plants at intervals (Broadbent & Martini, 1959).

DIRECT ATTACK ON THE INSECT VECTORS

Until recently, the outlook for the control of virus diseases of crops by attempting to eradicate the insect vectors by means of insecticides was not hopeful. Some recent work, however, by Broadbent and his co-workers (1956, 1958) have shown that good results can be obtained by this method in the control of potato viruses. It is not sufficient to kill the aphids which have been bred in a crop, they must be killed as they enter a crop, and this is now possible with the new persistent and systemic insecticides.

It has been shown by Broadbent *et al.* (1956), so far as potato viruses are concerned, that both contact and systemic insecticides will prevent the spread of the leaf-roll virus from infected plants within the crop. In the case of the non-persistent virus Y, however, insecticides can neither prevent its introduction nor its spread within the crop, although experiments suggest that this can be decreased. Thus, even if spraying did not prevent the degeneration of stocks when virus Y was present, it enabled them to be kept for 3 or 4 years in an area where they normally degenerate in 2 years. Insecticides can do little to prevent the introduction of virus into a crop, and these trials showed that when infective aphids land on a sprayed crop they can infect plants before they die. Furthermore, although non-infective aphids arriving upon a sprayed potato crop would be killed before they could acquire and transmit a persistent virus like that of leaf-roll, they can often acquire

and transmit a non-persistent virus, like potato virus Y, before dying.

BREEDING RESISTANT VARIETIES OF CROPS

One of the promising methods of control lies in the production of virus-immune or resistant varieties of plants. This is a long-range method and for it we must look to the plant breeder. Some success in this direction has already been achieved. Several good varieties of mosaic-resistant sugar-cane have been produced, known as the P.O.J. strains, and the substitution of these for susceptible varieties in most of the sugar-growing areas has reduced the disease to one of small importance, although at one time it threatened the very existence of the sugar-cane industry. Similarly with the sugar-beet in the U.S.A; at one time the curley-top disease was so serious that in large areas of the Union the growing of sugar-beet had to be abandoned. However, by the combination of a number of strains selected for resistance, varieties have been produced (U.S. Nos. 1, 33 and 34) which have a fair degree of resistance to curly-top and are reasonably satisfactory as regards sugar content, &c.

In England, many parts of Europe, and now in the U.S.A., virus yellows is also a serious disease of sugar-beet. This is a very difficult problem to tackle because no factor of resistance to the virus which might be used in breeding seems to exist either in the wild beet, *Beta maritima*, or in any varieties of the sugar-beet itself.

Strains of cotton of the Sakel type resistant to the leaf-curl disease have been evolved, and these seem to combine vigour and fruitfulness with a high degree of resistance.

The production of a virus-resistant variety of potato is a problem of the greatest importance, but the position is complicated by the new viruses which have been added to the number since the last edition of this book was published.

The choice before the plant breeder is to develop either tolerant or 'carrier' types or intolerant varieties. The

drawbacks to the carrier or tolerant types are, first, their liability to act as a source of infection to other more susceptible varieties of potato, and secondly, the fact that a second virus infection added to the carried virus produces a more severe disease than would otherwise be the case. The aim behind the development of intolerant varieties is to make them so susceptible to the virus or viruses in question that they are killed outright. Such varieties are said to be 'field-immune' since the virus is destroyed with its host and cannot spread further.

In the U.S.A. a potato seedling, No. 41956, has been produced which is not only resistant but appears to be actually immune from infection with potato virus X, the most widespread of all potato viruses.

Breeding for some form of resistance to virus X and the other important potato viruses, leaf-roll and virus Y, is being carried on in Scotland (Cockerham, 1958), U.S.A. (Ross, 1958), New Zealand (Hutton, 1952) and other centres. It was shown by Stelzner (1950) that an apparent immunity from virus X existed in the wild potato *Solanum acaule*, and breeding experiments with this and *S. demissum* are being carried out by Cockerham (1958). *S. stoloniferum* is field-immune to potato virus Y and the genetics of this resistance have been studied by Ross (1958).

CURE OF VIRUS-INFECTED PLANTS

Two methods of eliminating the virus from infected plants are considered here, first, inactivation of the virus by heat, and secondly by chemicals.

The first experiments on heat therapy were made by Kunkel (1936), who subjected peach trees, infected with peach yellows, little peach, red suture and rosette, to temperatures of 35° C. The trees were kept at this temperature for a fortnight or longer, and the time necessary was longer for large trees than for small; it was easier to destroy the virus in the top of the trees than in the roots. That the trees were actually cured of the disease is shown

by the fact that scions from the treated trees produced no disease when grafted onto a virus-free tree. Moreover, cured trees could be re-infected with the viruses, which shows there was no question of attenuation or masking of infection. Later, Kunkel (1941) showed that the virus of aster yellows could also be destroyed by heating the host plant, but the treatment could only be applied to certain plants such as periwinkle, *Vinca rosea* and *Nicotiana rustica*, which could survive being grown at 40° C. for 2 weeks. Since these pioneer experiments, plants infected with about thirty viruses have been cured by heat therapy, but only one or two examples can be given here and the reader is referred to a review by Kassanis (1957*a*) for an excellent survey of the whole subject. Ratoon stunt, a serious disease of sugar-cane in Queensland, is now controlled by exposing the setts for 2 hours in hot water at 50° C. In 1953 over 2,000 tons of cane were given the hot-water treatment in wire baskets, holding a ton at a time, immersed in special tanks (Hughes, 1954; Greenaway, 1954). Kassanis (loc. cit.) has shown that the leaf-roll virus is inactivated in potato tubers after 20 days at 36° C., but when potato plants similarly infected are treated in the same way both they and their tubers remain infected. Posnette & Cropley (1958) have carried out experiments on the heat inactivation of strawberry viruses in some twenty varieties of strawberry. Treatment, up to 50 days' duration, at 37° C. inactivated the mottle virus, though the time needed for permanent inactivation of the virus varied considerably. Elimination of crinkle virus, though effected in 9 days in the variety Huxley's Giant, required 30–50 days in other varieties. The yellow-edge virus appears more difficult to eradicate; some plants are apparently cured after 26 days but relapse more than a year later. Vein chlorosis virus is also as hard to eliminate as crinkle.

Budsticks from sweet cherry (variety Lambert) infected with necrotic rusty mottle virus were treated in a hot-water bath at 50° C. for 10, 13 and 15 minutes, and at 52° C. for 5, 8 and 10 minutes. Virus inactivation occurred in all

treatments. Seven of ten Lambert trees that received buds heated at 50° C. for 15 minutes and at 52° C. for 10 minutes survived at least 2 years (Nylands, 1959).

At the moment there is no well-established example of the practical control of a plant virus disease by means of a chemical acting on the virus in the host. The principle underlying the application of chemical therapeutics is that multiplication of a virus can be delayed by compounds which interfere with the nucleic acid metabolism. If nucleic acids are the most important part of viruses and the bases the most important part of nucleic acids, it seems reasonable to look for virus-inhibitory agents among synthetic analogues of those natural bases. For this reason Matthews (1954) used 8-azaguanine and found that when sprayed onto plants it had quite a marked effect on the spread of virus within the plant. It was found most effective against the viruses of lucerne and cucumber mosaic in the tobacco plant. Later, Matthews (1955) carried out experiments on the inhibition of development of turnip yellow mosaic virus, again using 8-azaguanine. These experiments suggested that, as with tobacco mosaic virus, the incorporation of the base into the nucleic acid of the turnip yellow mosaic virus renders a proportion of the virus particles incapable of initiating infection.

Thiouracil is another substance which inhibits to a certain extent the initial multiplication of several viruses in growing plants. There is no evidence, however, that it affects the virus content of systemically infected plants. Kassanis & Tinsley (1958) carried out some experiments on the effect of this substance on potato virus Y growing in normal tobacco tissue cultures. They succeeded in freeing the cultures of the virus by growing them for 3 weeks or more on media containing 100 mg./l of thiouracil. Progenies from these cultures were still free of detectable virus one year after the treatment.

Although the chemotherapy of plant virus diseases is only beginning, the results achieved so far are at least suggestive that some practical applications of the method

will be developed in the future. For a comprehensive survey of the chemotherapy of virus diseases the reader is referred to a review by Matthews & Smith (1955).

SPECIAL METHODS OF PROPAGATION

By taking advantage of the rate, or lack, of movement of a virus in a plant it is sometimes possible to propagate from tissues which are temporarily free of invading virus. This is a useful technique in cases of valuable plants or where it is desired to build up a virus-free clone of a particular variety.

For example, virus-free plants of dahlias infected with the virus of tomato spotted wilt may be obtained by taking cuttings from the tips of shoots as they arise from the tubers. At a time of rapid growth the movement of the virus fails to keep pace and there is often a few inches of tissue not yet reached by the virus (Holmes, 1948).

Since some viruses fail to invade the growing-point, the apical meristem may be cut off and grown in tissue culture (Morel & Martin, 1952, 1955). When large enough, the plantlets can be transferred to soil and a virus-free plant obtained. By this method potato plants of the variety King Edward have been obtained free of the paracrinkle virus with which all commercial stocks of this variety are infected. This is of considerable interest since no King Edward potato plant had previously been seen without the latent paracrinkle virus. All attempts made previously to eliminate the virus, by radiation or by heat treatment, had failed. By a similar technique 'Arran Victory' potatoes have been freed of virus S (Kassanis, 1957*b*).

REFERENCES

BROADBENT, L. (1958). 'The spread and control of plant viruses.' *Sci. Hort.* **13**, 1957–8.

BROADBENT, L., BURT, P. E. and HEATHCOTE, G. D, (1956). 'The control of potato virus diseases by insecticides.' *Ann. appl. Biol.* **44**, 256–73.

BROADBENT, L. HEATHCOTE, G. D. and MASON, E. C. (1958). 'An Essex farm trial on the insecticidal control of potato virus spread.' *Plant Path.* **7,** 53–55.

BROADBENT, L. and MARTINI, C. (1959). 'The spread of plant viruses.' *Advances in Virus Research,* **6,** 93–135.

CADMAN, C. H. (1956). 'Studies on the etiology and mode of spread of raspberry leaf curl disease.' *J. hort. Sci.* **31,** 111–18.

COCKERHAM, G. (1958). 'Experimental breeding in relation to virus resistance.' *Proc. 3rd Conf. Pot. Virus Dis.* Wageningen, pp. 199–203.

GREENAWAY, S. (1954). 'Notes on the hot water treatment of cane setts in the Mackay District, 1953.' *Proc. Queensland Soc. Sugar Cane Technol.* p. 201.

HOLMES, F. O. (1948). 'Elimination of spotted wilt from a stock of dahlia.' *Phyotpathology* (abstr.), **38,** 314.

HUGHES, C. G. (1954). 'Ratoon stunting disease of sugar-cane.' *Int. Sugar J.* **56,** 338.

HUTTON, E. M. (1952). 'Some aspects of virus Y resistance in the potato (*Solanum tuberosum*).' *Austral. J. agric. Res.* **3,** 362–71.

KASSANIS, B. (1957*a*). 'Effects of changing temperatures on plant virus diseases.' *Advances in Virus Research* **4,** 221–41.

(1957*b*). 'The use of tissue cultures to produce virus-free clones from infected potato varieties.' *Ann. appl. Biol.* **45,** 422–7.

KASSANIS, B. and TINSLEY, T. W. (1958). 'The freeing of tobacco tissue from potato virus Y by 2-thiouracil.' *Proc. 3rd Conf. Pot. Virus Dis.* Wageningen, 153–5.

KENNEDY, J. S. (1958). 'Physiological condition of the host plant and susceptibility to attack.' *Entomologia,* **1,** 50–65.

KUNKEL, L. O. (1936). 'Heat treatment for the cure of yellows and other virus diseases of peach.' *Phyto-pathology,* **26,** 809–30.

(1941). 'Heat cure of aster yellows in periwinkles.' *Amer. J. Bot.* **28,** 761–9.

LIMASSET, P. and CORNUET, P. (1949). 'Recherche du virus de la mosaique du tabac (*Marmor tabaci* Holmes) dans les meristèmes des plantes infectées.' *C.R. Acad. Sci. Paris*, **228,** 1971–2.

MATTHEWS, R. E. F. (1954). 'Effects of some purine analogues on tobacco mosaic virus.' *J. gen. Microbiol.* **10,** 521–32.

(1955). 'Infectivity of turnip yellow mosaic virus containing 8-azaguanine.' *Virology*, **1,** 165–75.

MATTHEWS, R. E. F. and SMITH, J. D. (1955). 'Chemotherapy of Viruses.' *Advances in Virus Research*, **3,** 51–148.

MOREL, G. and MARTIN, C. (1952). 'Guérison de dahlia atteints d'une maladie à virus.' *C.R. Acad. Sci. Paris*, **235,** 1324.

(1955). 'Guérison de pomme de terre atteints de maladies à virus.' *C.R. Acad. Agric. France*, **41,** 472.

MÜLLER, H. J. (1956). *Sitzber. deut. Akad. Landwirtsch. Berlin*, **5,** 1.

NYLANDS, G. (1959). 'Hot water treatment of Lambert cherry budsticks infected with necrotic rusty mottle virus.' *Phytopathology*, **49,** 157–8.

POSNETTE, A. F. and CROPLEY, R. (1958). 'Heat treatment for the inactivation of strawberry viruses.' *J. hort. Sci.* **33,** 282–8.

ROSS, H. (1956). 'Probleme der Resistenzzüchtung gegen Viruskrankheiten in Pflanzen.' *Arch. dtsch. LandwGes.* **17,** 95–108.

(1958). 'Inheritance of extreme resistance to virus Y in *Solanum stoloniferum* and its hybrids with *Solanum tuberosum*.' *Proc. 3rd Conf. Pot. Virus Dis.* Wageningen, 204–11.

STELZNER, G. (1950). 'Virusresistenz der Wildkartoffeln.' *Z. Pflzücht.* **29,** 135–58.

VAN DER PLANCK, J. E. and ANDERSSEN, E. E. (1944). 'Kromnek disease of tobacco.' *Farming S. Africa*, **28,** 391–4.

APPENDIX

Some Common Viruses and their Indicator Plants

Partly after Hollings (1956, 1957)

Virus disease	*Indicator plant*	*Local symptoms*	*Systemic infection*
Anemone Brown Ring	*Chenopodium amaranticolor*	Chlorotic spots, developing reddish-brown margins	—
Anemone Mosaic	*Chenopodium amaranticolor*	Chlorotic spots, becoming red-rimmed rings	—
Arabis Mosaic	*Cucumis sativus*	Faint chlorotic lesions and ringspots	Mottle, severe stunting and subsequent death of plant
Aspermy	*Chenopodium amaranticolor*	Numerous chlorotic dots, some becoming brown-rimmed rings	—
Barley False Stripe	*Chenopodium amaranticolor*	Yellowish spots, some extending along veins. Leaves yellow and absciss	—
Bean Yellow Mosaic	*Chenopodium amaranticolor*	Few faint chlorotic lesions tending to become necrotic	—
	Crotalaria spectabilis	Small brownish black lesions, solid or ring-type	Severe stem and tip necrosis
Beet Latent Virus	*Vigna sinensis*	Red-brown lesions, enlarging slightly, but not spreading. Old lesions have white centre	Occasional necrosis of veins and stem

Beet Mosaic	*Chenopodium amaranticolor*	Bold semi-necrotic spots which enlarge and spread along veins. Some veinal necrosis	Necrotic flecks and necrosis of smaller veins. Leaf puckering. Plants grow away
Beet Yellows	*Chenopodium capitatum*	Very severe symptoms and stunting (Russell)	
Belladonna Mosaic	*Phaseolus vulgaris*. Canadian Wonder	Necrotic, distinct and non-spreading lesions	—
Broad Bean Mottle	*Chenopodium amaranticolor*	ex *Vicia faba* – numerous tiny chlorotic dots	—
Brome Mosaic Virus	*Chenopodium hybridum*	Discrete necrotic local lesions (Rochow, 1959)	Not systemic
Cabbage Black Ringspot	*Chenopodium amaranticolor*	Chlorotic spots, becoming red-rimmed rings	—
Carnation Mottle	*Chenopodium amaranticolor*	Numerous yellow-green lesions	Occasional veinal flecks
Carnation Ringspot	*Chenopodium amaranticolor*	Numerous tiny greenish-white dots	One isolate induced severe twisting, yellow flecks and apical die-back
Carnation Vein Mottle	*Chenopodium amaranticolor*	Few lesions with central necrotic spot and diffuse chlorotic halo	—
Chrysanthemum D	*Chenopodium amaranticolor*	Few faint chlorotic spots, developing red margins and spreading along veins. May become necrotic wedge	—

APPENDIX—*continued*

Virus disease	*Indicator plant*	*Local symptoms*	*Systemic infection*
Chrysanthemum Stunt	Chrysanthemum Blazing Gold	—	Diffuse yellow bands along veins
Cucumber Mosaic (most strains)	*Chenopodium amaranticolor*	Bright green to yellow-green dots. Similar to Aspermy	—
(spinach strain)	*Chenopodium amaranticolor*	Bright yellow dots, becoming white with red-brown margins	—
Cucumber 4	*Cucumis sativus*	—	Mottle with yellow and white star-like spots
Cymbidium Mosaic	*Chenopodium amaranticolor*	Small white dots, becoming larger	—
	Datura stramonium	Numerous distinct red necrotic lesions	—
Dandelion Yellows	*Chenopodium amaranticolor*	Faint chlorotic spots, sometimes becoming necrotic	—
Dock Mosaic	*Chenopodium amaranticolor*	Faint, diffuse chlorotic lesions	—
Henbane Mosaic	*Chenopodium amaranticolor*	Faint chlorotic lesions, becoming pale green spots or rings	—
Hydrangea Ringspot	*Chenopodium amaranticolor*	Numerous chlorotic dots with necrotic centres	—
Iris Mosaic	*Chenopodium amaranticolor*	Occasional small faint chlorotic dots. Unreliable	—

Lettuce Mosaic	*Chenopodium amaranticolor*	Bright yellow-green spots, becoming larger	Yellowish spots or veinal flecks. Leaf curling. Plants stunted
	Chenopodium urbicum	Lesions	—
	Gomphrena globosa	Distinct lesions	—
Lucerne Mosaic	*Chenopodium amaranticolor*	Numerous faint semi-necrotic dots	Chlorotic flecks, streaks and dots. Severe leaf-curling and stunting of plant
Narcissus Stripe	*Chenopodium amaranticolor*	Irregular diffuse chlorotic spots (inoculation from flowers)	—
Pea Mosaic	*Chenopodium amaranticolor*	Chlorotic spots	Chlorotic spots and veinal flecks, becoming bright yellow vein-clearing
Pea Mottle	*Phaseolus vulgaris*	Light green areas becoming brownish-red necrotic. Sometimes slight necrosis instead of lesions	Mild mosaic
Pelargonium Leaf-Curl	*Chenopodium amaranticolor*	Buff dots, usually developing chlorotic halo	Lesions
Pelargonium Ring-spot (*P. paltatum*)	*Pelargonium zonale*	—	Yellowish spots and rings 10–12 months after grafting
Privet Mosaic	*Chenopodium amaranticolor*	Few indistinct necrotic dots	—

APPENDIX—*continued*

Virus disease	*Indicator plant*	*Local symptoms*	*Systemic infection*
Radish Mosaic	*Nicotiana glutinosa*	Faint chlorotic lesions	—
Rose Mosaic	*Cyanopsis psoraloides*	Distinct lesions	—
	Cucumis sativus	Faint chlorotic spots	Chlorosis followed by necrosis of growing-point. Lethal
Tobacco Broken Ringspot	*Phaseolus vulgaris.* Canadian Wonder	Pale chlorotic areas followed by small necrotic ringspots	Blistering and puckering of leaves with necrosis of veins
Tobacco Mosaic and Tomato Mosaic	*Chenopodium amaranticolor*	Chlorotic dots and flecks	—
Tobacco Necrosis	*Chenopodium amaranticolor*	Numerous pale fawn necrotic dots; becoming brown necrotic rings from some isolates	—
Tobacco Rattle	*Nicotiana tabacum*	Large necrotic lesions	Severe necrotic streaks on stem and leaf veins. Necrotic lesions on leaves which become puckered and deformed
Tobacco Ringspot	*Chenopodium amaranticolor*	Tiny ringspots	One isolate produced chlorotic spots and veinal flecks with leaf-buckling

Tobacco Severe Etch	*Chenopodium amaranticolor*	Chlorotic spots	—
Tomato Black Fleck (serologically related to TMV)	*Chenopodium amaranticolor*	Numerous faint, irregular, semi-necrotic lesions	Yellowish-grey veinal flecks and spots, becoming necrotic. Leaf curling. Plants killed in winter
Tomato Black Ring	*Chenopodium amaranticolor*	Dull yellow lesions becoming tiny yellow rings and flecks	Yellowish veinal flecks and spots. Distortion
Tomato Bushy Stunt	*Vigna sinensis*	Small pale lesions becoming red with a pale centre as they enlarge	—
Tomato Spotted Wilt	*Chenopodium amaranticolor*	Numerous chlorotic dots, becoming tiny semi-necrotic ring-spots	—
Tulip White Streak	*Nicotiana tabacum*	Very large transparent necrotic lesions with irregular brown margins	Occasional necrosis of petiole and stem
Turnip Crinkle	*Chenopodium amaranticolor*	Numerous chlorotic dots	—
Turnip Yellow Mosaic	*Brassica chinensis*	Chlorotic or reddish local lesions. Unreliable	Pronounced vein-clearing followed by bright yellow or white mosaic with dark green areas. White flower-break

Index