PLANT VIROLOGY

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PREFACE

As in many other areas of biology, there has been rapid growth over the past few years in our knowledge of plant viruses and the diseases they cause. Thus there was a substantial need for a new text covering all aspects of the subject.

This book was written primarily for graduate students in plant pathology, plant virology, general virology, and microbiology and for teachers and research workers in these fields. I hope that it will also prove useful as a reference work for those in disciplines related to plant virology—molecular biologists, biochemists, plant physiologists, and entomologists.

I have attempted to cover, to some degree at least, all aspects of the subject, a difficult task in view of the wide range of disciplines involved. There is a brief historical account of the development of plant virology in the first chapter, but the general approach is not a historical one. Those interested will find this aspect well covered in earlier texts.

Topics dealt with include the structure of viruses and viral components; the replication of viruses; their macroscopic, cytological, and biochemical effects on the host plant; the nature of virus mutation; relationships with invertebrate vectors; and a discussion of ecology and control. Throughout I have attempted to indicate how progress in any particular area has been dependent on the development and application of appropriate experimental methods. Specific details of methodology have not been given since these are available elsewhere.

The subject has grown to the extent that it would be impossible to quote all papers on any given topic in a book of this size. In general I have referred to important early papers and to the most important or most suitably illustrative recent papers. From these the reader should be able to gain rapid access to the literature on any relevant topic.

In a text on a subject that draws on a wide range of scientific disciplines, I believe that illustrative material is most important, particularly for students or newcomers to the field. For this reason I have gone to some pains, and have had the support of many colleagues, in selecting graphs and photographs to highlight and supplement the text.

In certain areas, particularly the molecular biology of viral replication, our knowledge of plant viruses lags behind that of animal and bacterial

viruses. I have therefore drawn on information about these viruses where it seemed appropriate to set the stage for considering more fragmentary facts about plant viruses.

One recent development that created problems was the discovery that many diseases previously thought to be caused by unstable viruses are very probably caused by mycoplasma-like organisms. Although, in general, I have not included diseases in which the probability of a mycoplasma-like organism being involved is high, one chapter on agents causing virus-like diseases is devoted mainly to a consideration of such organisms in plant disease. Other recent work of considerable general interest has resulted in the discovery that several plant viruses have their genetic material divided up between two or more particles. Thus I have devoted a chapter to the consideration of defective virus particles, dependent viruses, and multiparticle viruses.

I have followed the Commonwealth Mycological Institute list of "Plant Virus Names" (Martyn, 1968). I have not attempted to deal with individual viruses or virus diseases in any systematic or comprehensive way, so that the list of "Plant Virus Names" should be regarded as a valuable companion book for the present text, especially for those interested in the tremendous amount of literature on the plant pathological aspects of virus diseases.

In the last chapter I have outlined the various viewpoints regarding nomenclature and classification. Since, from the long-term point of view, at least, classification of viruses must take origins into consideration, some space is given to speculation on the origins of viruses.

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INTRODUCTION

As with any other branch of experimental science, the development of our knowledge of plant viruses has been closely dependent on the techniques available for their study. In this chapter, I will briefly outline these developments, and summarize the more important aspects of recent work.

Although virus diseases in plants were not recognized as distinct from other kinds of infectious disease until about 70 years ago, there are much earlier records of what now can be seen to be virus infections. For example, the Frontispiece includes a plate from Parkinson's Herbal (1656) showing several tulip flowers with color-breaking typical of that caused by virus infection. At this period, such blooms were prized as special varieties. In the latter part of the nineteenth century, the idea that infectious disease was caused by microbes was well established, and filters were available which would not allow the known bacterial pathogens to pass. Mayer (1886) described a disease of tobacco which he called *Mosaikkrankheit*. He showed that the disease could be transmitted to healthy plants by inoculation with extracts from diseased plants. Iwanowski (1892) showed that sap from tobacco plants displaying the disease described by Meyer was still infective after it had been passed through a bacteria-proof filter candle and was sterile as far as bacteria were concerned. This work did not attract much attention until it was repeated by Beijerinck (1898). Baur (1904) showed that the infectious variegation of *Abutilon* could be transmitted by grafting, but not by mechanical inoculation. Beijerinck and Baur used the term virus in describing the causative agents of these diseases to contrast them with bacteria. The term virus had been used as more or less synonymous with bacteria by earlier workers. As more diseases of

this sort were discovered the unknown causative agents came to be called "filterable viruses." Between 1900 and 1935, many plant diseases thought to be due to filterable viruses were described, but considerable confusion arose because adequate methods for distinguishing one virus from another had not been developed.

One important step forward was the recognition that some viruses could be transmitted from plant to plant by insects. For example, Smith and Boncquet (1915) confirmed earlier suggestions that sugar beet curly top disease could be transmitted by the leafhopper *Eutettix tenella* (Baker), and showed that a single insect from an infected plant could induce the disease in a healthy one by only 5 minutes feeding. However, they did not at that time put forward the view that sugar beet curly top was due to a virus.

The original criterion of a virus was an infectious entity that could pass through a filter with a pore size small enough to hold back all known cellular agents of disease. However, diseases were soon found that had viruslike symptoms not associated with any pathogen visible in the light microscope, but which could not be transmitted by mechanical inoculation. With such diseases, the criterion of filterability could not be applied. The infectious nature was established by graft transmission and sometimes by insect vectors. Thus it came about that certain diseases of the yellows and witches'-broom type, such as aster yellows, came to be considered as due to viruses on quite inadequate grounds. Only very recently, critical examination by electron microscopy and the use of inhibitory drugs have given strong indications that a number of these so-called virus diseases are really caused by mycoplasma-like agents (Doi *et al.*, 1967).

During most of the period between 1900 and 1935, attention was focused on description of diseases, both macroscopic symptoms and cytological abnormalities as revealed by light microscopy, and on the host ranges and methods of transmission of the disease agents. Rather ineffective attempts were made to refine filtration methods in order to define the size of viruses more closely. These were almost the only aspects of virus disease that could be studied with the techniques that were available. The influence of various physical and chemical agents on virus infectivity was investigated, but methods for the assay of infective material were primitive. Holmes (1929) showed that the local lesions produced in some hosts following mechanical inoculation could be used for the rapid quantitative assay of infective virus. This technique enabled properties of viruses to be studied much more readily and paved the way for the isolation and purification of viruses a few years later.

Until about 1930, there was serious confusion by most workers of the

diseases produced by viruses and the viruses themselves. This was not surprising, since virtually nothing was known about the viruses except that they were very small. Smith (1931) made an important contribution that helped to clarify this situation. Working with virus diseases in potato he realized the necessity of using plant indicators—plant species other than potato, that would react differently to different viruses present in potatoes. Using several different and novel biological methods to separate the viruses, he was able to show that many potato virus diseases were caused by a combination of two viruses with different properties, which he named X and Y. Virus X was not transmitted by the aphid *Myzus persicae* (Sulz.), while virus Y was. In this way, he obtained virus Y free of virus X. Both viruses could be transmitted by needle inoculation, but Smith found that certain Solanaceous plants were resistant to virus Y. For example, by needle inoculation of the mixture to *Datura stramonium*, he was able to obtain virus X free of virus Y. Furthermore, Smith observed that virus X from different sources fluctuated markedly in the severity of symptoms it produced in various hosts. To quote from Smith (1931), "There are two factors, therefore, which have given rise to the confusion which exists at the present time with regard to potato mosaic diseases. The first is the dual nature, hitherto unsuspected, of so many of the potato virus diseases of the mosaic group, and the second is the fluctuation in virulence exhibited by one constitutent, i.e., X, of these diseases."

Another discovery that was to become important was Beale's (1928) recognition that plants infected with tobacco mosaic contained a specific antigen. Gratia (1933a,b) showed that plants infected with different viruses contained different specific antigens. Chester (1936b) showed that different strains of tobacco mosaic virus (TMV) and potato virus X could be distinguished serologically, and drew up the first serological classification of plant viruses (Chester, 1937). Serological methods could be used to obtain a rough estimate of virus concentration. Before TMV was isolated, Chester (1935) estimated from serological end point measurements on sap from infected tobacco plants compared with end points on some purified nonvirus antigens that TMV must exist in tobacco sap in concentrations of at least 0.1–1.0 mg/ml. (The concentration in fully infected plants was subsequently shown to be about 2–4 mg/ml.)

The high concentration at which certain viruses occur in infected plants and their relative stability turned out to be of crucial importance in the first isolation and chemical characterization of viruses, because methods for extracting and purifying proteins were not highly developed. In 1926, the first enzyme urease was isolated, crystallized, and identified as a protein (Sumner, 1926). The isolation of others soon followed. In the early 1930's, workers in various countries began attempting to isolate and purify

plant viruses using methods similar to those that had been used for enzymes. Following detailed chemical studies suggesting that the infectious agent of TMV might be a protein, Stanley (1935) announced the isolation of this virus in an apparently crystalline state. At first Stanley (1935, 1936) considered that the virus was a globulin containing no phosphorus. About the same time Best (1936a) noted that a globulin-like protein having virus activity was precipitated from infected leaf extracts when they were acidified. Bawden *et al.* (1936) described the isolation from TMV-infected plants of a liquid crystalline nucleoprotein containing nucleic acid of the pentose type. They showed that the particles were rod-shaped, thus confirming the earlier suggestion of Takahashi and Rawlins (1932) based on the observation that solutions containing TMV showed anisotropy of flow. Bernal and Fankuchen (1937) applied the technique of X-ray analysis to the purified preparations. They obtained accurate estimates of the width of the rods and showed that the needle-shaped bodies produced by precipitating the virus with salt were regularly arrayed in only two dimensions and, therefore, were better described as paracrystals than as true crystals. The isolation of other rod-shaped viruses, and spherical viruses which formed true crystals, soon followed. All were shown to consist of protein and pentose nucleic acid.

Electron microscopy and X-ray crystallography have been the major techniques used to explore the architecture of virus particles. Early electron micrographs (Kausche *et al.*, 1939) confirmed that TMV was rod-shaped and provided approximate dimensions, but they were not particularly revealing because of the lack of contrast between the virus particles and the supporting membrane. The development of shadow-casting with heavy metals (Müller, 1942; Williams and Wycoff, 1944) greatly increased the usefulness of the method for determining the overall size and shape of virus particles. However, the coating of metal more or less obscured structural detail. With the development of high resolution microscopes and of negative staining in the 1950's, electron microscopy has become an important tool for studying virus substructure.

From a comparative study of the physicochemical properties of the virus nucleoprotein and the empty viral protein shell found in turnip yellow mosaic virus (TYMV) preparations, Markham (1951) concluded that the RNA of the virus must be held inside a shell of protein, a view that has since been amply confirmed for this and other viruses by X-ray crystallography. Crick and Watson (1956) suggested that the protein coats of small viruses are made up of numerous identical subunits arrayed either as helical rods or as a spherical shell with cubic symmetry. Subsequent X-ray crystallographic and chemical work has confirmed this view. Caspar and Klug (1962) formulated a general theory that delimited the

possible numbers and arrangements of the protein subunits forming the shells of the smaller isodiametric viruses. Our recent knowledge of the larger viruses with more complex symmetries and structures has come from electron microscopy using negative-staining and ultrathin-sectioning methods.

Until about 1948, most attention was focused on the protein part of viruses. There are probably several reasons for this. Quantitatively, the protein made up the larger part of the virus preparations. Enzymes which carried out important functions in cells were known to be proteins, and knowledge of pentose nucleic acids was rudimentary. No function was known for them in cells, and they generally were thought to be small molecules. This was because it was not recognized that these nucleic acids were very susceptible to hydrolysis by acid, alkali, and by enzymes that commonly contaminate virus preparations.

Markham and Smith (1949) isolated TYMV and showed that purified preparations contained two classes of particle, one an infectious nucleoprotein with about 35% of RNA, and the other an apparently identical protein particle which contained no RNA and which was not infectious. This result clearly indicated that the RNA of the virus was important for biological activity. Analytical studies (Markham and Smith, 1951, 1952a, b,c; Dorner and Knight, 1953) showed that different viruses have characteristically different base compositions, and that related viruses have similar base compositions. About this time it came to be realized that viral RNA's might be considerably larger than had been thought.

The experiments of Hershey and Chase (1952), showing that when *Escherichia coli* was infected by a bacterial virus, the viral DNA entered the host cell while most of the protein remained outside, emphasized the importance of the nucleic acids in viral replication. Harris and Knight (1952) showed that 7% of the threonine could be removed enzymically from TMV without altering the biological activity of the virus, and that inoculation with such dethreonized virus gave rise to normal virus with a full complement of threonine. A synthetic analog of the normal base guanine, 8-azaguanine, when supplied to infected plants was incorporated into the RNA of TMV and TYMV, replacing some of the guanine. The fact that virus preparations containing the analog were less infectious than normal virus (Matthews, 1953d, 1954, 1955) gave further experimental support to the idea that viral RNA's were important for infectivity. However, it was the classic experiments of Gierer and Schramm (1956), Fraenkel-Conrat and Williams (1955), and Fraenkel-Conrat (1956) that demonstrated the infectivity of naked TMV RNA and the protective role of the protein coat. Subsequent work has shown that for viruses like TMV,

the RNA is present as one strand, and a single cleavage in this strand leads to inactivation of the virus particle.

Until a few years ago, all the plant viruses that had been examined contained single-stranded RNA as their genetic material. It now is known that wound tumor (Black and Markham, 1963) and rice dwarf viruses (Miura *et al.*, 1966) contain double-stranded RNA, while cauliflower mosaic virus contains double-stranded DNA (Shepherd *et al.*, 1968b; Shepherd, personal communication). Viruses containing single-stranded DNA will probably be found among plant viruses also, since they are known among those infecting bacteria and animals.

The volumes occupied by particles of the known plant viruses cover roughly a 300-fold range from about 2.6×10^3 $m\mu^3$ for satellite virus to about 8.0×10^5 $m\mu^3$ for lettuce necrotic yellows and similar viruses. The molecular weight (MW) of plant virus nucleic acids whose size is known varies approximately 20-fold in a range from 0.4×10^5 daltons for satellite virus to 10^7 daltons for wound tumor virus, and others will probably be found that are larger than this.

The full sequence of bases has not yet been determined for any viral RNA. The technical problems involved and the generally large size of viral RNA's made it seem that such sequence determination might be many years away. However, the full sequence of 120 bases in 5 S ribosomal RNA from *E. coli* has been established recently (Brownlee *et al.*, 1968). This RNA, like viral RNA, contains no unusual bases to assist in the identification of fragments. Thus the possibility of determining the full sequence for satellite virus RNA (containing about 1200 nucleotides) does not seem so remote.

Knowledge about the replication of plant viruses in the host cell is much less advanced than for animal and bacterial viruses. The main reasons for this are technical ones. So far, it has not been possible to develop a plant system in which all cells can be infected simultaneously and in which the subsequent replicative events occur more or less synchronously. The development of such a system would be a major step forward. A second difficulty with plant viruses is the extreme inefficiency of the available methods for the inoculation of cells. For many viruses that can be transmitted thereby, mechanical inoculation requires some 10^4–10^6 virus particles to be applied to the leaf surface for each cell that is to become infected. For the multiparticle viruses discussed below the number is much higher. This compares with a 1:1 ratio for many bacterial viruses and ratios approaching this for some animal systems.

Despite these difficulties some progress has been made. Double-stranded viral RNA has been isolated from leaves in which TMV and TYMV were replicating (Mandel *et al.*, 1964; Shipp and Haselkorn, 1964; Burdon *et*

al., 1964) but the details of how such structures function in the replication of viral RNA at present must be inferred from experiments with similar bacterial and animal viruses. We know that in leaves infected with TYMV viral protein synthesis can continue when RNA synthesis is suppressed by an inhibitor (Francki and Matthews, 1962a), but again the details of the process of viral protein synthesis must be inferred largely from results with animal or bacterial systems.

Many bacterial and animal viruses cause death of the cells in which they replicate. This also can happen in plants, but many viruses can occur in high concentration in plant cells without marked deleterious effects. In a systemically infected plant, virus infects dividing cells near the growing tip of the plant and may reach a high concentration in cells that yet have to undergo many cycles of cell division before the mature organ is formed. In such situations virus synthesis appears to be a process as closely regulated as, say, the synthesis of ribosomes. The nature of such regulation is quite obscure.

The full sequence of 158 amino acids in the coat protein of TMV is known (Anderer *et al.*, 1960; Tsugita *et al.*, 1960; Wittmann and Wittmann-Liebold, 1966) and that of many naturally occurring strains and artificially induced mutants has been determined. This work made an important contribution to establishing the universal nature of the genetic code and to our understanding of the chemical basis of mutation.

The knowledge that the genetic code is a series of triplets of bases in the nucleic acid, each specifying a single amino acid in a protein, has led to the realization that most viruses carry substantially more genetic information than is required to specify the protein or proteins found in the virus particle. For example, many plant viruses contain an RNA molecule with a MW $\simeq 2 \times 10^6$ daltons. This is sufficient to code for about 5 to 8 average-sized proteins besides the coat protein. These proteins are presumably required for virus replication and are synthesized in the infected cell. By analogy with work on animal and bacterial viruses, one of these proteins is probably a virus-specific RNA synthetase. The isolation and characterization of these noncoat proteins is a challenge for the future.

Many studies have been made on the effects of virus infection on such metabolic processes of the host as respiration and photosynthesis, and on the concentration of various normal metabolites and macromolecules. The connection between changes in such processes or components and virus replication probably will remain obscure until we know more about the activities of the various proteins formed in infected cells under the influence of the viral genome.

Regarding the assembly of virus coat proteins and nucleic acids into virus particles, the idea that the assembly of the protein shells of small