Chapter 1

Introduction

SUMMARY
This chapter describes the history of plant virology noting that in several instances it has led the whole of virology in revealing various concepts. It then discusses how plant viruses differ from other disease-causing agents leading on to the definition of a virus. It concludes by giving a brief description of the layout of the book.

I. HISTORICAL (reviewed by van der Want and Dijkstra, 2006)

The scientific investigation of plant diseases now known to be caused by viruses did not begin until the late nineteenth century. However, there are much earlier written and pictorial records of such diseases. The earliest known written record describing what was almost certainly a virus disease is a poem in Japanese written by the Empress Koken in 752 A.D. and translated by T. Inouye as follows:

In this village
It looks as if frosting continuously
For, the plant I saw
In the field of summer
The color of the leaves were yellowing

The plant, identified as *Eupatorium lindleyanum*, has been found to be susceptible to TLCV,[1] which causes a yellowing disease (Osaki et al., 1985).

In Western Europe in the period from about 1600 to 1660, many paintings and drawings were made of tulips that demonstrate flower symptoms of virus disease. These are recorded in the herbals of the time (e.g., Parkinson, 1656) and some of the earliest in the still-life paintings of artists such as Ambrosius Bosschaert. During this period, blooms featuring such striped patterns were prized as special varieties leading to the phenomenon of "tulipomania" (see Blunt, 1950; Pavord, 1999; Thompson, 2007). The trade in infected tulip bulbs resulted in hyperinflation with bulbs exchanging hands for large amounts of money or goods (Box 1.1).

[1] Acronyms of virus names are shown in Appendix D.

One of the earliest written accounts of an unwitting experimental transmission of a virus is that of Lawrence (1714). He described in detail the transmission of a virus disease of jasmine by grafting. This description was incidental to the main purpose of his experiment, which was to prove that sap must flow within plants. The following quotation from Blair (1719) describes the procedure and demonstrates, rather sadly that, even at this protoscientific stage, experimenters were already indulging in arguments about priorities of discovery.

The inoculating of a strip'd Bud into a plain stock and the consequence that the Stripe or Variegation shall be seen in a few years after, all over the shrub above and below the graft, is a full demonstration of this Circulation of the Sap. This was first observed by Mr. Wats at Kensington, about 18 years ago: Mr. Fairchild performed it 9 years ago; Mr. Bradly says he observ'd it several years since; though Mr. Lawrence would insinuate as if he had first discovered it.

Blair, 1719

In the latter part of the nineteenth century, the idea that infectious disease was caused by microbes was well established, and filters were available that would not allow the known bacterial pathogens to pass. Mayer (1886) (Figure 1.1A) described a disease of tobacco that he called *Mosaikkrankheit*. He showed that the disease could be transmitted to healthy plants by inoculation with extracts from diseased plants. lwanowski (1892) showed that sap from tobacco plants displaying the disease described by Mayer was still infective after it had been passed through a bacteria-proof filter candle. This work did not attract much attention until it was repeated by Beijerinck (1898) (Figure 1.1B) who described the infectious agent as "contagium vivum fluidum" (Latin for contagious living fluid) to distinguish it from contagious corpuscular agents (Figure 1.1C). The centenary of Bejerinck's discovery, which was considered to be the birth of virology, was marked by several publications and celebratory meetings (see Zaitlin, 1998; Bos, 1999; 2000; Harrison and Wilson, 1999; Scholthof et al., 1999; van Kammen, 1999).

The word "virus" is of Latin origin where it described "a slimy liquid, poison, noxious substance, stench or offensive taste." It was first used in English in the late

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BOX 1.1 Tulipomania

Tulips were introduced into the Netherlands in the late sixteenth century. There was great interest in bulbs that produced "broken" colored flowers for which a rapidly expanding market appeared leading to hyperinflation.

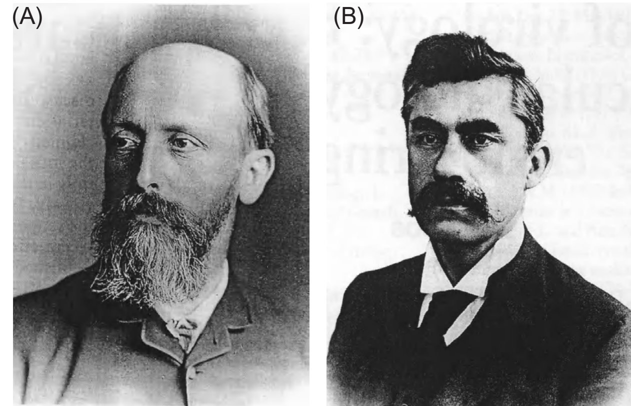
Semper Augustus tulip with flower color break (one of the most favored varieties)

One bulb cost 1000 Dutch florins (guilders) in 1623 and by 1635, 6000 florins. To understand the value of this one Viceroy tulip bulb, it was exchanged for goods of the value of almost 2400 florins made up of:

4 tons of wheat (448 florins)	4 barrels of beer (3 florins)
8 tons of rye (558 florins)	2 barrels of butter (192 florins)
4 fat oxen (480 florins)	1000 lbs cheese (120 florins)
8 fat pigs (240 florins)	1 bed with accessories (100 florins)
12 fat sheep (120 florins)	1 silver goblet (60 florins)
2 hogsheads of wine (70 florins)	

By 1636, there was much speculation and futures were being taken out on these bulbs. In early 1637, one bulb was valued at 10,000 florins but a few weeks later the bubble burst and many people were left bankrupt.

It was not until the 1920s that the viral etiology of tulip flower breaking was revealed and that the symptoms were caused by an aphid-transmitted potyvirus.



(C)

FIGURE 1.1 (A) Adolf Eduard Mayer (1843–1942); (B) Martinus Willem Beijerinck (1851–1931; (C) Page from lab journal of W.M. Beijerinck from 1898 relating to TMV. *(A) and (B) courtesy of the historical collection, Agricultural University, Wageningen, Netherlands; (C) (© Kluyver Institute) Courtesy Curator Kluyver Laboratory Collection, Delft University of Technology.*

fourteenth century to mean a poison and was first associated with disease in the mid-eighteenth century. It became used for the filterable infectious agent in the early twentieth century.

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." The papers by Mayer, Iwanowski, Beijerinck, and Baur have been translated into English by Johnson (1942).

The development of plant, and other, virology can be considered to have gone through six major (overlapping) ages. The first two, *Prehistory* and *Recognition* of viral entity are described above. These were then followed by the *Biological* age between 1900 and 1935 in which there was the recognition that plant viruses were transmitted by insects and that some of these viruses multiplied in, and thus were pathogens of, insects in a manner similar to some viruses of vertebrates. The *Biochemical/Physical* age started in the early 1930s. The high concentration at which certain viruses occur in infected plants and their relative stability was crucial in their first isolation and chemical characterization, because methods for extracting and purifying proteins were not highly developed. In parallel with these biochemical studies, physical studies in the late 1930s using X-ray analysis and electron microscopy confirmed that TMV had rod-shaped particles and obtained accurate estimates of the size of the rods. The fifth age, the *Molecular* age, started in about 1960 when the full sequence of 158 amino acids in the coat protein of TMV was determined and has developed into an understanding of the detailed composition of viral genomes and how they replicate. In the current sixth age, which I am terming the *Viromics* age, the detailed interactions between the viral, plant host and invertebrate vector are being elucidated. The finding from these studies are throwing light not only on how viruses cause disease and plant "fight" back against them but also on a range of aspects of how uninfected plants function.

Between 1900 and 1935 (the biological age), there was considerable confusion about many plant diseases thought to be due to filterable viruses that were being described because adequate methods for distinguishing one virus from another had not yet been developed. 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 virus-like symptoms not associated with any pathogen visible in the light microscope, but that could not be transmitted by mechanical inoculation. With such diseases, the criterion of filterability could not be applied, but their infectious nature was established by graft transmission and sometimes by insect vectors. Thus, certain diseases of the yellows and witches' broom type, such as aster yellows, came to be attributed to viruses on quite inadequate grounds. Many such diseases are now known to be caused by mycoplasma-like organisms (phytoplasma and spiroplasmas), and a few, such as ratoon stunting of sugarcane, by bacteria.

An important practical step forward was the recognition that some viruses could be transmitted from plant to plant by insects. Fukushi (1969) records the fact that in 1883 a Japanese rice grower transmitted what is now known to be RDV by the leafhopper *Recelia dorsalis*. However, this work was not published in any available form and so had little influence. Kunkel (1922) first reported the transmission of a virus by a planthopper; within a decade, many insects were reported to be virus vectors.

During most of the period between 1900 and 1935, attention was focused on the 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.

It was not surprising that until about 1930, there was serious confusion by most workers regarding the diseases produced by viruses and the viruses themselves 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, which would react differently to different viruses present in potatoes. Using several different and novel biological methods to separate the viruses, he showed that certain 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*, whereas 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 regards 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 constituent, 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 (1933) showed that plants infected with different viruses contained different specific antigens. Chester (1935, 1936) demonstrated that different strains of TMV and PVX could be distinguished serologically. He also showed that serological methods could be used to obtain a rough estimate of virus concentration.

Since Fukushi (1940) first demonstrated that RDV could be passed through the egg of a leafhopper vector for many generations, there has been great interest in the possibility that some viruses may be able to replicate in both plants and insects. It is now well established that plant viruses in the families *Rhabdoviridae*, *Bunyaviridae* and *Reoviridae* and the *Tenuivirus* and *Marafivirus* genera multiply in their insect vectors as well as in their plant hosts.

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 1930s, workers in various countries began attempting to isolate and purify plant viruses using methods similar to those that had been used for enzymes, opening up the biochemical/biophysical age. 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. Bawden et al. (1936) described the isolation from TMV-infected plants of a liquid crystalline nucleoprotein containing pentose-type nucleic acid. 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. Best (1936) noted that a globulin-like protein having virus activity was precipitated from infected leaf extracts when they were acidified, and in 1937 he independently confirmed the nucleoprotein nature of TMV (Best, 1937).

Electron microscopy and X-ray crystallography were the major techniques used in early work to explore virus structure, and the importance of these methods has continued to the present day. Bernal and Fankuchen (1937) applied X-ray analysis to purified preparations of TMV. 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 of spherical viruses that formed true crystals, soon followed. All were shown to consist of protein and pentose nucleic acid.

Early electron micrographs (Kausche et al., 1939) confirmed that TMV was rod-shaped and provided approximate dimensions, but the structure was not particularly well revealed 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 1950s (see Chapter 3, Section I, C, 3), electron microscopy became 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 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 (see Chapter 3, Section IV, A). 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 the viruses. Quantitatively, the protein made up the larger part of virus preparations. Enzymes that 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 RNA is very susceptible to hydrolysis by acid, by 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 that contained no RNA and that was not infectious. This result clearly indicated that the RNA of the virus was important for biological activity. Analytical studies (e.g., Markham and Smith, 1951) showed that the RNAs of different viruses have characteristically different base compositions while those of related viruses are similar. About this time, it came to be realized that viral RNAs might be considerably larger than had been thought.

The experiments of Hershey and Chase (1952), which demonstrated that when *Escherichia coli* was infected by a bacterial virus, the viral DNA entered the host cell while most of the protein remained outside, emphasizing the importance of the nucleic acids in viral replication. Harris and Knight (1952) showed that 7% of the threonine could

be removed enzymatically 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, 1953) gave further experimental support to the idea that viral RNAs 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. Genome types other than RNA were first noted in the late 1960s. Shepherd et al. (1968) reported that CaMV has a dsDNA genome and the ssDNA genomes of Geminiviruses was described by Goodman (1977a,b), and Harrison et al. (1977). For several years it was though the Caulimoviruses replicated like dsDNA animal viruses (DNA>DNA) but in 1983 it was recognized that their replication was by reverse transcription, resembling in several respects that of animal-infecting retroviruses (see Chapter 7, Section VII) (Guilley et al., 1983; Hull and Covey, 1983; Pfeiffer and Hohn, 1983).

The first amino acid sequence of a protein (insulin) was established in 1953. Soon after that, the full sequence of 158 amino acids in the coat protein of TMV was determined (Anderer et al., 1960; Tsugita et al., 1960; Wittmann and Wittmann-Liebold, 1966); the sequences of many naturally occurring strains and artificially induced mutants was also determined at about the same time. 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.

Brakke (1951, 1953) developed density gradient centrifugation as a method for purifying viruses. This has been an influential technical development in virology and molecular biology. Together with a better understanding of the chemical factors affecting the stability of viruses in extracts, this procedure has allowed the isolation and characterization of many viruses. The use of sucrose density gradient fractionation enabled Lister (1966, 1968) to discover the bipartite nature of the TRV genome. Since that time, density gradient and polyacrylamide gel fractionation techniques have allowed many viruses with multipartite genomes to be characterized. Their discovery, in turn, opened up the possibility of carrying out genetic reassortment experiments with plant viruses (Lister, 1968; van Vloten-Doting et al., 1968).

Density gradient fractionation of purified preparations of some other viruses has revealed noninfectious nucleoprotein particles containing subgenomic RNAs. Other viruses have been found to have associated with them satellite viruses or satellite RNAs that depend on the "helper" virus for some function required during replication. With all of these various possibilities, it is in fact rather uncommon to find a purified virus preparation that contains only one class of particle.

The 1960s can be regarded as the decade in which electron microscopy was a dominant technique in advancing our knowledge about virus structure and replication. Improvements in methods for preparing thin sections for electron microscopy allowed completed virus particles to be visualized directly within cells. The development and location of virus-induced structures within infected cells could also be studied. It became apparent that many of the different groups and families of viruses induce characteristic structures, or viroplasms, in which the replication of virus components and the assembly of virus particles take place. Improved techniques for extracting structural information from electron microscope images of negatively stained virus particles revealed some unexpected and interesting variations on the original icosahedral theme for the structure of "spherical" viruses. These are described in detail in Chapter 3.

There were further developments in the 1970s. Improved techniques related to X-ray crystallographic analysis and a growing knowledge of the amino acid sequences of the coat proteins allowed the three-dimensional structure of the protein shells of several plant viruses to be determined in molecular detail.

For some decades, the study of plant virus replication had lagged far behind that of bacterial and vertebrate viruses. This was mainly because there was no plant system in which all the cells could be infected simultaneously to provide the basis for synchronous "one-step growth" experiments. However, following the initial experiments of Cocking (1966), Takebe and colleagues developed protoplast systems for the study of plant virus replication (reviewed by Takebe, 1977). Although these systems had significant limitations, they greatly increased our understanding of the processes involved in plant virus replication. Another important technical development has been the use of *in vitro* protein-synthesizing systems, such as that from wheat germ, in which many plant viral RNAs act as efficient messengers. Their use allowed the mapping of plant viral genomes by biochemical means to begin.

During the 1980s, major advances were made on improved methods of diagnosis of virus diseases, centering on serological procedures and on methods based on nucleic acid hybridization. Since the work of Clark and Adams (1977), the ELISA technique has been developed with many variants for the sensitive assay and detection of plant viruses. Monoclonal antibodies against TMV were described by Dietzgen and Sander (1982) and Briand et al. (1982). Since that time, there has been a very rapid growth in the use of monoclonal antibodies for many kinds of plant virus research and for diagnostic purposes.

The late 1970s and the 1980s also saw the start of application of the powerful portfolio of molecular biological techniques to developing other approaches to virus diagnosis, to a great increase in our understanding of the organization and strategy of viral genomes, and the development of techniques that promise novel methods for the control of some viral diseases.

The use of nucleic acid hybridization procedures for sensitive assays of large numbers of samples has been greatly facilitated by two techniques: (i) the ability to prepare double-stranded cDNA from a viral genomic RNA and to replicate this in a plasmid grown in its bacterial host, with the batches of cDNA labeled radioactively or with nonradioactive reporter molecules to provide a sensitive probe; and (ii) the dot blot procedure, in which a small sample of a crude plant extract containing virus is hybridized with labeled probe as a spot on a sheet of nitrocellulose or other material.

The understanding on the genome organization and functioning of viruses has come from the development of procedures whereby the complete nucleotide sequence of viruses with RNA genomes can be determined opening up the molecular age. Of special importance has been the ability to prepare *in vitro* infectious transcripts of RNA viruses derived from cloned viral cDNA (Ahlquist et al., 1984). This has allowed techniques such as site-directed mutagenesis to be applied to the study of genome function. Nucleotide sequence information has had, and continues to have, a profound effect on our understanding of many aspects of plant virology, including: (i) the location, number, and size of the genes in a viral genome; (ii) the amino acid sequence of the known or putative gene products; (iii) the molecular mechanisms whereby the gene products are transcribed; (iv) the putative functions of a gene product, which can frequently be inferred from amino acid sequence similarities to products of known function coded for by other viruses; (v) the control and recognition sequences in the genome that modulate expression of viral genes and genome replication; (vi) the understanding of the structure and replication of viroids and of the satellite RNAs found associated with some viruses, (vii) the molecular basis for variability and evolution in viruses, including the recognition that recombination is a widespread phenomenon among viruses and that viruses can acquire host nucleotide sequences as well as genes from other viruses; and (viii) the beginning of a taxonomy for viruses that is based on evolutionary relationships.

In the early 1980s, it seemed possible that some plant viruses or regulatory sequences derived from them, when suitably modified by the techniques of gene manipulation, might make useful vectors for the introduction of foreign genes into plants. This, together with a detailed understanding of virus structure, is now leading to the use of plant viruses and their sequences in the pharmaceutical and other industries such as nanotechnology (see Chapter 15).

In the early decades of the last century, attempts to control virus diseases in the field were often ineffective. They were mainly limited to attempts at general crop hygiene, roguing of obviously infected plants, and searches for genetically resistant lines. Developments since this period have improved the possibilities for control of some virus diseases. The discovery of two kinds of soil-borne virus vectors (fungi, Grogan et al., 1958; nematodes, Hewitt et al., 1958) opened the way to possible control of a series of important diseases. Increasing success has been achieved with a range of crop plants in finding effective resistance or tolerance to viruses.

Heat treatments and meristem tip culture methods have been applied to an increasing range of vegetatively propagated plants to provide a nucleus of virus-free material that then can be multiplied under conditions that minimize reinfection. Such developments frequently have involved the introduction of certification schemes. Systemic insecticides, sometimes applied in pelleted form at the time of planting, provide significant protection against some viruses transmitted in a persistent manner by aphid vectors. Diseases transmitted in a nonpersistent manner in the foregut or on the stylets of aphids have proved more difficult to control. It has become increasingly apparent that effective control of virus disease in a particular crop in a given area usually requires an integrated and continuing program involving more than one kind of control measure. However, such integrated programs are not yet in widespread use.

Cross-protection (or mild-strain protection) is a phenomenon in which infection of a plant with a mild strain of a virus prevents or delays infection with a severe strain. The phenomenon has been used with varying success for the control of certain virus diseases, but the method has various difficulties and dangers. Powell-Abel et al. (1986) considered that some of these problems might be overcome if plants could be given protection by expression of a single viral gene. Using recombinant DNA technology, they showed that transgenic tobacco plants expressing the TMV coat protein gene either escaped infection following inoculation or showed a substantial delay in the development of systemic disease. These transgenic plants expressed TMV coat protein mRNA as a nuclear event. Seedlings from self-fertilized transformed plants that expressed the coat protein showed delayed symptom development when inoculated with TMV. Thus, a new approach to the control of virus diseases emerged. Since these experiments, the phenomenon has been shown to be widespread and two basic types of protection have been recognized—one based on the expression of the gene product and the other being nucleic acid based. Both of these are leading to potentially economically useful protection against specific viruses in several crops but are raising various nonscientific and ethical questions about the acceptability of this approach.

The late 1980s and 1990s were a period where molecular biological techniques were applied to a wide range of

aspects of plant virology. As well as those areas described above, reverse genetics was, and still is, being used to elucidate the functions of viral genes and control sequences. This approach together with others such as yeast systems for identifying interacting molecules and to transform plants to express viral genes and coupled with the ability to label viral genomes in such a manner that their sites of function within the cell is revealing the complexities of the interactions between viruses and their hosts. The advances in plant genome sequencing are identifying plant genes that interact with viruses. A major advance in the late 1990s arising, to a great extent, from the work on transformation of plants with viral sequences was the recognition that plants have a generic defense system against "foreign" nucleic acids. Coupled with this is the identification of viral genes that suppress this defense system (see Chapter 9).

Over the last decade the rapid advances in technology are opening up other approaches, such as metagenomics and transcriptomics (reviewed by Mokili et al., 2012; Radford et al., 2012). Metagenomics is the analysis of genetic material extracted from whole organisms, communities of organisms, or environmental samples. The usual approach is to sequence the genetic material by high-throughput sequencing and subject the resulting sequences to bioinformatic analyses. In this way many new, previously undescribed viral sequences are being discovered (e.g., Muthukumar et al., 2007; Roossinck et al., 2010; Ng et al., 2011; Molina et al., 2012).

A major challenge has been to understand how infection with an entity containing a small genome encoding few proteins can cause often devastating disease in plants. Major advances in understanding the interactions between viruses, their plant hosts and invertebrate vectors have been made in the last 10–15 years, especially from high-throughput or deep-sequencing and transcriptomics. It is becoming apparent that the genomes of viruses, hosts, and vectors interact with each other in integrated manners which ultimately lead to a plant becoming infected or not infected. These interactions involve the vector being attracted to an infected plant, acquiring the virus, feeding on a healthy plant and transmitting the virus, and the virus replicating in that plant, overcoming host resistance and causing the plant to show symptoms. In all these stages the genomes of the three parties involved interact—I am terming this VIROMICS. These interactions are revealing many features of normal plant functions and is a major area where plant virology is contributing to the understanding these functions offering the potential of new approaches to crop protection.

In recent years, considerable progress has been made in the development of a stable and internationally accepted system for the classification and nomenclature of viruses. One thousand and sixteen plant viruses have been placed in three orders, 21 families, and 92 genera. The 21 virus families and most (but not all) of the genera are very distinctive entities. They possess clusters of physical and biological properties that often make it quite easy to allocate a newly isolated virus to a particular family or genus. The rapidly expanding information on nucleotide sequences of viruses infecting plants, invertebrates, vertebrates, and microorganisms is emphasizing, even more strongly than in the past, the essential unity of virology. The time is therefore ripe for virologists to consider more grouping into higher taxa.

These advances in our understanding of plant viruses, how they function, and how this knowledge can be applied to their control has resulted in a burgeoning of papers on the subject. This is illustrated in Figure 1.2 which shows

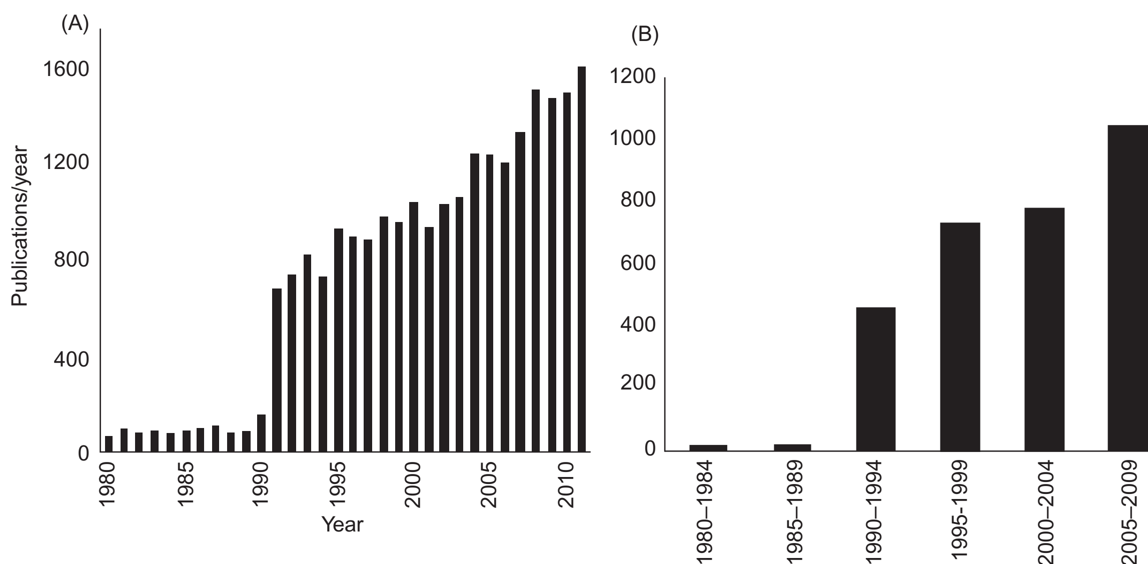


FIGURE 1.2 (A) Annual number of publications on "Plant Virus" from 1980 to 2011. (B) Publications on "Plant Virus + Symptoms" over 5-year periods from 1980 to 2009.

the numbers of papers that have Plant Virus and Plant Virus + Symptoms in their titles, abstracts, and key words. Figure 1.2A illustrates the general interest in plant viruses with a sudden increase in 1991, possibly driven by studies on virus sequence (33% of the papers) and molecular aspects of resistance (21% of the papers). Figure 1.2B shows the gathering interest since 1990 in molecular aspects of symptom production.

More details of the historical development of plant virology are discussed by Zaitlin and Palukaitis (2000) and a collection on seminal papers on TMV, which have led many of the conceptual advances is published by Scholthof et al., 1999. Hull et al. (1989) provide a useful directory and dictionary of viruses and terms relating to virology.

II. DEFINITION OF A VIRUS

When defining a virus we have to consider not only plant viruses but also those that infect organisms in other kingdoms, such as vertebrates and bacteria. In the size of their nucleic acids, viruses in general range from a monocistronic mRNA in the satellite virus of tobacco necrosis virus (STNV) to a genome larger than that of the smallest cells (Figure 1.3). Before attempting to define what viruses are, we must consider briefly how they differ from cellular parasites on the one hand and transposable genetic elements on the other. The three simplest kinds of parasitic cells are the mycoplasmas, the *Rickettsiae*, and the *Chlamydiae*.

Mycoplasmas and related organisms are not visible by light microscopy. Cells are 150–300 nm in diameter with a bilayer membrane, but no cell wall. They contain ribosomes and DNA. They replicate by binary fission, and some that infect vertebrates can be grown *in vitro*. Their growth is inhibited by certain antibiotics.

The *Rickettsiae*, for example, the agent of typhus fever, are small, nonmotile bacteria, usually about 300 nm in diameter. They have a cell wall, plasma membrane, and cytoplasm with ribosomes and DNA strands. They are obligate parasites and were once thought to be related to viruses, but they are definitely cells because (i) they multiply by binary fission, and (ii) they contain enzymes for ATP production.

The *Chlamydiae*, for example, the agent causing psittacosis, include the simplest known type of cell. They are obligate parasites and lack an energy-generating system. They have two phases in their life cycle. Outside the host cell they exist as infectious *elementary bodies* about 300 nm in diameter. These bodies have dense contents, no cell wall, and are specialized for extracellular survival. The elementary body enters the host cell by phagocytosis. Within 8 h it is converted into a much larger noninfectious *reticulate body* which is bounded by a bilayer membrane derived from the host. The reticulate body divides by

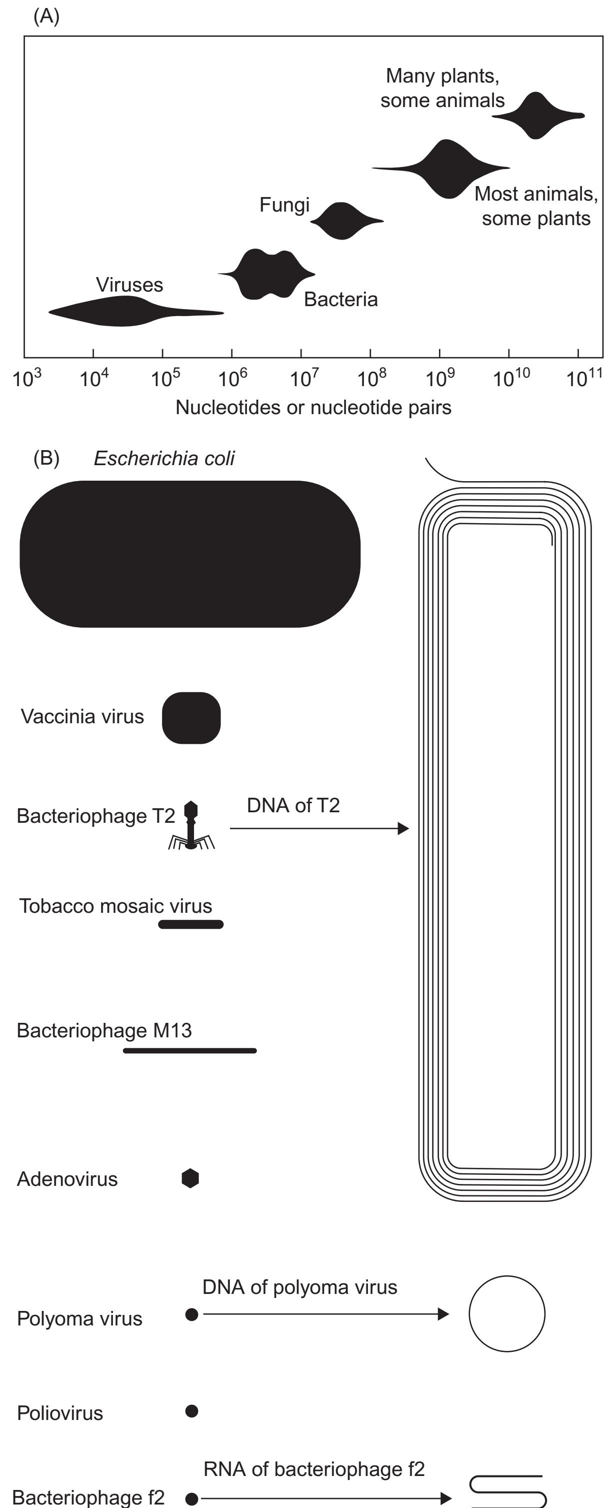


FIGURE 1.3 Size comparisons of different organisms. (A) Organisms classified according to genome size. The vertical axis gives an approximate indication of relative numbers of species (or viruses) within the size range of each group. (B) Size comparisons between a bacterium, several viruses, and the viroid. *(A) Modified from Hinegardner (1976) with permission of the publishers. (B) From Diener (1999) with permission of the publisher.*

binary fission within this membrane, giving thousands of progeny within 40–60 h. The reticulate bodies are converted to elementary bodies, which are released when the host cell lyses.

There are several criteria that do not distinguish all viruses from all cells. These include:

i. Size: some pox viruses are bigger than the elementary bodies of *Chlamydiae*.
ii. Nature and size of the genome: many viruses have dsDNA like that of cells, and in some the DNA is bigger than in the *Chlamydiae*.
iii. A rigid cell envelope is absent in viruses and mycoplasmas.
iv. Growth outside a living host cell does not occur with viruses or with many groups of obligate cellular parasites, for example, *Chlamydiae.*
v. An energy-yielding system is absent in viruses and *Chlamydiae.*
vi. Complete dependence on the host cell for amino acids, etc., is found with viruses and some bacteria.

There are four related criteria that do appear to distinguish all viruses from all cells:

i. Lack of a continuous membrane separating viral parasite and host during intracellular replication. Cellular parasites that replicate inside a host cell appear always to be separated from host cell cytoplasm by a continuous bilayer membrane. As described in Chapter 7, the replicating complexes of many viruses are contained within membranous structure, but these have a pore or neck connecting them to the rest of the cytoplasm.
ii. Absence of a protein-synthesizing system in viruses.
iii. Genome is either RNA or DNA but not both.
iv. Replication of viruses is by synthesis of a pool of components, followed by assembly of many virus particles from the pool. Even the simplest cells replicate by binary fission.

Plasmids are autonomous extrachromosomal genetic elements found in many kinds of bacteria. They consist of closed circular DNA. Some can become integrated into the host chromosome and replicate with it. Some viruses infecting prokaryotes have properties like those of plasmids and, in particular, an ability to integrate into the host cell chromosome. However, viruses differ from plasmids in the following ways:

i. Normal viruses have a particle with a structure designed to protect the genetic material in the extracellular environment and to facilitate entry into a new host cell.
ii. Virus genomes are highly organized for specific virus functions of no known value to the host cell, whereas plasmids consist of genetic material often useful for survival of the cell.
iii. Viruses can cause death of cells or disease in the host organism but plasmids do not.

We can now define a virus as follows: *A virus is a set of one or more nucleic acid template molecules, either RNA or DNA, normally encased in a protective coat or coats of protein or lipoprotein, that is able to organize its own replication only within suitable host cells. It can usually be horizontally transmitted between hosts. Within such cells, virus replication is (i) dependent on the host's protein-synthesizing machinery, (ii) organized from pools of the required materials rather than by binary fission, (iii) located at sites that are not separated from the host cell contents by a continuous lipoprotein bilayer membrane, and (iv) continually giving rise to variants through various kinds of change in the viral nucleic acid.*

To be identified positively as a virus, an agent must normally be shown to be transmissible and to cause disease in at least one host. However, the *Cryptovirus* group of plant viruses is an exception. Viruses in this group rarely cause detectable disease and are not transmissible by any mechanism except through the seed or pollen.

The structure and replication of viruses in general have the following features:

i. The nucleic acid may be DNA or RNA and single- or double-stranded. If the nucleic acid is single-stranded it may be of positive or negative sense (positive sense has the sequence that would be used in an mRNA for translation to give a virus-coded protein).
ii. The mature virus particle may contain polynucleotides other than the genomic nucleic acid.
iii. Where the genetic material consists of more than one nucleic acid molecule, each may be housed in a separate particle or all may be located in one particle.
iv. The genomes of viruses vary widely in size, encoding between 1 and about 250 proteins. Plant viral genomes are at the small end of this range, encoding between 1 and 12 proteins. The virus-coded proteins may have functions in virus replication, in virus movement from cell to cell, in virus structure, in overcoming host defense and in transmission by invertebrates or fungi.
v. Viruses undergo genetic change. Point mutations occur with high frequency as a result of nucleotide changes brought about by errors in the copying process during genome replication. Other kinds of genetic change may be due to recombination, reassortment of genome pieces, loss of genetic material, or acquisition of nucleotide sequences from unrelated viruses or the host genome.
vi. Enzymes specified by the viral genome may be present in the virus particle. Most of these enzymes are concerned with nucleic acid synthesis.
vii. Replication of many viruses takes place in distinctive virus-induced regions of the cell, known as viroplasms.

viii. Some viruses share with certain nonviral nucleic acid molecules the property of integration into host cell genomes and translocation from one integration site to another.

ix. A few viruses require the presence of another virus for their replication.

A question that is frequently asked is "are viruses alive?" There are various definitions of a living organism and the most widely accepted one being: "A living organism has cellular structure and is manifest by growth through metabolism, reproduction and the power of adaptation to the environment through changes originating internally." While viruses reproduce and adapt, they are not cellular and do not metabolize—they rely on their host cell metabolism. Thus, technically they are not living organisms and the term "virus life cycle" should not be used; "virus infection cycle" describes the processes of infection of a healthy host from an infected host and "virus replication cycle" describes the making of a new virus particle from an input particle.

III. VIRUSES AND KOCH'S POSTULATES

To be identified positively as a virus, an agent must normally be shown to be transmissible and to cause disease in at least one host. One of the basic tenets of pathology is that to prove that a disease is caused by a certain infectious agent one must fulfill Koch's postulates (Koch, 1891) (Table 1.1). These postulates were devised for bacteria and have proved difficult, if not impossible, to fulfill for viruses. Various modifications have been suggested to account for specific properties of viruses (see Rivers, 1937) (Table 1.1), but even these are not always possible to use for viruses. For instance, plant cryptoviruses rarely cause detectable disease and are not transmissible by any mechanism except through the seed or pollen. Usually, until recently it has been satisfactory to show clear association of the viral genome sequence with the disease after eliminating the possibility of joint infection with another virus. However, further advances in technologies have necessitated revisiting the criteria for applying Koch's postulates to the recognition of new viruses. Microbial genetics and molecular cloning now permit the routine isolation of specific genes from a variety of viruses; this has been addressed by Falkow (1988). The finding of large numbers of potentially new viruses by the use of metagenomics is raising yet further problems with Koch's postulates *sensu stricto*; this is addressed by Mokili et al. (2012)

IV. THIS EDITION

This edition follows many of the features of previous editions but has been reorganized to take account of the greater understanding of how viruses function and interact with their hosts. The first chapters (1–5) describe the basic features of viruses, their classification, the symptoms they cause, how they are purified, what they are made of and the structure of their particles. The next three chapters (6–8) recount how viruses express their genetic information, replicate themselves, vary and have evolved. This is followed by several chapters (9–12) discussing the interactions between viruses and their hosts and vectors in disease transmission and manifestation. The next three chapters (13–15) deal with the detection and control of plant viruses and biotechnical applications of virus particles and their sequences. In the last chapter I discuss how the genomes of viruses, their hosts and vectors interact, looking towards the future of an important area of plant virology. It is hoped that this will form a logical sequence and will reveal the breadth and dynamism of the subject.

In such a dynamic subject, there has been a plethora of publication over the last 10 years since the previous edition (see Figure 1.3). In many cases, I have referred to review papers on specific topics where the original papers on that topic can be found. I have retained many of the older references from the previous edition as these describe

TABLE 1.1 Koch's Postulates for Bacteria and Viruses

Bacteria	Viruses[a]
1. Demonstrate that the agent is regularly found in the diseased host	**1.** Isolation of virus from diseased host
2. Cultivate the agent on a suitable medium	**2.** Cultivate virus in experimental host or host cells
3. Reproduce the disease in the host by reintroducing the cultured agent	**3.** Prove lack of larger pathogens
4. Reisolate the agent from the artificially infected host	**4.** Produce comparable disease in original host species or in related ones
	5. Reisolate the virus

[a]*Rivers (1937).*

phenomena and results that can assist in the interpretation of the new phenomena that are being unveiled. The older references also put a perspective on the subject which can be swamped by the new "in vogue" topics.

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