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A history of plant virology

Brief Review

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Summary. This review traces developments in plant virus research from its very beginning in the eighties of the 19th century until the present day.

Starting with the earliest research, which gave a clue as to the existence of a pathogen different from the then known bacteria and fungi, the subsequent topics in plant virus research are highlighted, including the spread of plant viruses in nature and their relationships with possible vectors.

In the course of more than a century, macroscopical and (sub)microscopical studies gave way to those with a molecular dimension, thanks to the development of sophisticated molecular-biological techniques and information technology. As a result an insight has been gained into both the molecular characteristics of plant viruses and various resistance mechanisms in plants.

Introduction

In order to describe historical developments in the field of plant virology[2], the present authors had to make a choice from the very comprehensive literature published in the past century. They also had to restrict themselves regarding the topics to be dealt with in particular. The subjective element in such selections is reflected by the use of the indefinite article "A" in the title. To treat the material in an orderly fashion, various aspects of plant virology have been considered separately in their historical development. The authors trust that this review will

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[2]A historical overview was published by Zaitlin and Palukaitis in 2000. These authors stress developments in molecular plant virology and genetic engineering; they also attempt to predict the direction plant virology will take in the future [211][3].

give the reader some idea as to what has been achieved in plant virology in a time-span of a little over a hundred years[3].

Connotation of the term "virus"

Being of Latin origin "virus" had the meaning of slimy liquid, poison, stench, and – in addition – even offensive taste, clearly something unfavourable[4]. According to the Oxford English Dictionary, in the 18th century, "virus" got the meaning of a morbid principle or poisonous substance produced in the body as the result of some disease, especially one capable of being introduced into other persons or animals by inoculation or otherwise and of developing the same disease in them.

Jenner, the English physician who studied the incidence of smallpox at the end of the 18th century, called the liquid obtained from pox pustules in cattle "vaccine virus"[5]. With this exudate he inoculated persons to protect them against smallpox (variola). At that time, various theories were advanced to explain the origin of contagious diseases[6]. According to one theory, unfavourable factors could upset a postulated equilibrium present in healthy individuals, leading to the "spontaneous generation" of entities that could spread to other individuals of the population and give rise to disease.

A real breakthrough in the study of contagious diseases came with the pioneering work of Louis Pasteur and Robert Koch[7]. They showed that micro-organisms (germs) were responsible for the occurrence of infectious disorders in man and animals. Pasteur was convinced that virus was one of these autonomous micro-organisms. He therefore specified virus as *contagium vivum* or *contagium vivum fixum*: a living infectious entity of corpuscular, cellular nature.

Koch's work included the isolation of bacteria from diseased organs. He introduced the pure culture technique by using solid media. After having established microscopically that the isolated bacteria were identical with those in the diseased organs, he was able to reproduce the disease by introducing the isolated bacteria into a healthy specimen. He then formulated the conditions a micro-organism has to fulfil to be regarded as a pathogen (Koch's Postulates). Until 1833, the year in which F. Unger published his book entitled "Exanthemen der Pflanzen", plant diseases were still considered to be caused by aberrations in the chemical processes in plants due to adverse conditions[8]. As a result of these aberrations, spontaneous development of organisms – mostly fungi – would then occur. However, by the

[3]Numbers in brackets in the text refer to "literature cited" at the end of this overview.

[4]Webster's Third New International Dictionary III pp 2556, 1981. Oxford English Dictionary, Complete text reproduced micrographically II pp 3640, 1971.

[5][167: 112].

[6][167: 1, 29–30].

[7]C.E. Dolman wrote the biography of R. Koch including his work (Dictionary of Scientific Biography 7: 420–435, 1973) and G.L. Geison of L. Pasteur (Dictionary of Scientific Biography 10: 350–416, 1974).

[8]Unger is cited in Sorauer [169, p 14].

time bacteria became known to be the incitants of diseases in humans and animals, botanists discovered a connection between certain plant diseases and pathogenic bacteria [85, p 17]. At the end of the 19th century, Burrill described a bacterium causing pear blight, and Wakker showed a bacterium to be the causal agent of yellow disease of hyacinth.

In the last quarter of that century, a technique was used to free liquids from micro-organisms. This technique, developed by Chamberland, an associate of Pasteur, consisted of filtration of the liquids through a candle-shaped filter of unglazed porcelain mounted on a suction flask [44]. The size of the pores of the filter-candle was such that coarse particles, including bacteria and other micro-organisms, could not pass and were left behind. The size of the pores depended on the temperature to which the clay used for the filter had been exposed: the higher the temperature the smaller the pore size, and at too high a temperature the pores would even disappear and no liquids would pass [126]. As at that time it was generally believed that micro-organisms were unable to pass such filters, the discovery that the causal agent of a plant disease was present in the filtrate puzzled the microbiologists. In 1882, Adolph Mayer had described this mysterious disease of tobacco in a preliminary publication written in Dutch, and he had named it "mozaïkziekte" (mosaic disease) because of the variegated leaves with light green and dark green patches [121]. In his publication of 1886 (in German), Mayer stated that he was unable to show the presence of a fungus in the diseased plants, hence he expected a bacterium to be the causal organism [122]. However, although he was able to transmit the contagious agent by mechanical means, he could not attribute the disease to any bacteria. Interestingly, he casually remarked already in his paper of 1882 that perhaps a soluble, enzyme-like infectious principle was involved. However, Mayer did not elaborate on that idea and he still stuck to the concept of a pathogenic bacterium.

Mayer did not carry out filtration experiments with sap from mosaic-diseased plants. It was Ivanovskij[9] who published in 1892 that the incitant of tobacco mosaic could pass a filter of the type described above [93]. He assumed that a toxic substance was excreted by bacteria or that the bacteria were so small that they could pass through the filter pores [94, 95].

Beijerinck was the first to suspect that the incitant of tobacco mosaic, which he referred to as "spot disease", was an entity completely different from any micro-organism. In 1898 he published the results of filtration experiments with sap from diseased tobacco plants[10]. Being unaware of Ivanovskij's earlier results – as Beijerinck pointed out in a later paper [15] – he stated that the pathogen could pass filter pores [14]. As he could not detect bacteria or other disease-inciting

[9]The transliteration of this Russian name may differ from instance to instance. The present authors chose the spelling as indicated.

[10]November 26, 1898, Beijerinck reported in a lecture to the Royal Netherlands Academy of Sciences in Amsterdam on the filtration experiments. Extensive reports by Beijerinck appeared in 1898 and 1899 in German [14,15] and in 1900 in French [16]. Essentially, they represent the same scientific material.

organisms, he named the incitant *contagium vivum fluidum* [14]. In this way, he characterized it as a contagious, living (it reproduced in inoculated plants) and soluble (not corpuscular) entity. It is important to note that Beijerinck thus made a clear distinction between the filterable agent and a *contagium vivum fixum* as coined by Pasteur for pathogenic micro-organisms. Beijerinck stored the filtered infective sap for three months. Although no bacterial growth was observed, the sap was still infectious. With a small amount of virus (the term he used for the infectious material) many leaves could be infected. Because the sap of one of those leaves was capable of infecting many leaves, Beijerinck concluded that multiplication of the virus must have taken place.

Ivanovskij preferred the term *contagium solutum* over Beijerinck's *contagium fluidum*, as the latter literally meant "in a liquid form" which he did not consider correct for this infectious entity [95]. The year 1898 is marked by another historic event. In that year a research committee – established by the German government to study the foot-and-mouth disease, a serious cattle disorder – published its work. According to the third report of this committee, written by Loeffler and Frosch, the contagious entity (called virus) was found to pass a "Kieselguhr" filter, comparable to a porcelain filter [115], but made of infusorial earth instead of porcelain clay [126]. The filtrate was shown to be free of micro-organisms. The committee considered these results of great importance as some other animal and human pathogens, unlike bacteria, had been shown to be unable to grow on artificial media. In the fourth report, Loeffler stated that the foot-and-mouth disease agent could not pass a Kitasato filter, a type of filter resembling porcelain filters but with very small pores [114]. Therefore, the conclusion was drawn that the relevant virus was corpuscular and not dissolved. However, later it was established that the virus of this cattle disease was indeed a filterable agent [64]. To distinguish agents of this kind from pathogenic micro-organisms they were often referred to as filterable viruses. When the presence of infectious agents shown to be transmitted by grafting could not be established in the filtrate of sap of diseased plants, the word "filterable" was gradually dropped, and the pathogen was simply referred to as "virus". Henceforth, the term virus had acquired the restricted meaning of a pathogen of minute dimensions, unable to grow on artificial media. However, this definition of virus proved to be inadequate, as in 1967 it was reported that mycoplasma-like organisms, now referred to as phytoplasmas, occurred in the phloem of plants affected by witches' broom, formerly thought to be a virus disease [62]. Mycoplasma-like organisms resemble viruses in many respects as they are much smaller than bacteria and, most often, they cannot be grown on artificial media. Moreover, they are transmitted by grafting and by leafhoppers[11]. A few years later, the presence of a rickettsia-like organism, another very small pathogen, was demonstrated in the xylem of grapevine affected by Pierce's disease and in the xylem of roots of peach with phony disease [77, 92].

[11] Raychaudhuri and Varma presented an example of advances made in this field [147].

In 1967, yet another type of pathogen was discovered in potato plants showing symptoms of spindle tuber disease. This minute infectious agent resembled an RNA virus. But because it lacked a protein coat it was called a viroid[12].

Search for the nature of viruses

In studies of the intrinsic properties of viruses, the causal agent of tobacco mosaic, designated as tobacco mosaic virus (TMV), was used as a model because of its stability, relatively high concentration in sap of infected plants, and easy transmissibility. Since 1927, various investigations have revealed the intrinsic virus properties. Support for the concept of the proteinaceous nature of a plant virus came from a completely different discipline. In 1927, Dvorak injected experimental animals with sap from mosaic-diseased and healthy potato plants, and one year later, Helen Purdy Beale did the same with the sap from mosaic-diseased and healthy tobacco plants[13]. In both experiments, the sap from diseased plants contained a serologically active component (antigen) which was found missing in the healthy controls. Gratia showed that plants infected with different viruses also contained different antigens [80, 81]. Soon, the conclusion was drawn that the viruses themselves, like proteins, acted as antigens. Thus, a new way to characterize viral infections was found. It led to the development of serological tests to establish the presence of viruses in crops [196]. In the meantime, Vinson and Petre started physicochemical experiments [200, 201]. They precipitated the virus in plant sap using safranin or lead acetate. From these precipitates they acquired virus suspensions that were still infectious. It was Stanley who perfected the methods developed earlier, and, using a large quantity of TMV-containing plant sap, he obtained a suspension which he described as "a crystalline protein possessing the properties of tobacco mosaic virus" [170–172]. In other words, Stanley had produced the virus in a shape that bore resemblance to crystals. On dissolution of the crystals, the suspension obtained was infectious, also after repeated recrystallizations. Stanley explicitly mentioned that the suspension with the protein – most likely a globulin – did not contain any phosphorus or carbohydrates, and he considered the purified suspension to consist of protein only.

An even greater landmark was reached in 1936, one year after Stanley's first publication on the isolation of TMV, when Bawden and co-workers reported that in purified TMV suspensions a small amount of phosphorus (approximately 0.5%) and 2.5% carbohydrates were invariably present [11]. The latter two components were isolated as nucleic acid of the ribose type. Soon thereafter, Bawden and

[12]Diener and Raymer reported that the incitant of potato spindle tuber has the properties of a free nucleic acid [57, 58]. Later, Diener proposed the term "viroid" to designate this kind of incitant [56]. Diener remarked that this term had earlier been put forward by E. Altenburg (1946), The "viroid" theory in relation to plasmagenes, viruses, cancer and plastids. Amer Natur 80: 559–567; however, this theory did not meet any response.

[13]P(urdy) Beale referred to her first paper of 1928 and to the work of M. Dvorak [142].

Pirie described the TMV suspension as a liquid crystalline nucleoprotein with rod-shaped constituent particles [10].

The conclusion that TMV was a nucleoprotein fomented the old controversy originating from Beijerinck's publication in 1898, between biologists who considered TMV to be a living organism and chemists for whom the virus was just a nonliving chemical substance. One of the reasons why this controversy lasted for such a long time was that, so far, TMV had not been visualized. Indirect evidence that viruses are not contagious fluids, but corpuscular entities came from observations made by Takahashi and Rawlins and by Bawden and co-workers. The former authors exposed diluted partially purified sap from mosaic-diseased tobacco plants to polarized light by placing it between crossed Nicol prisms [175]. The virus-containing suspension showed birefringence, indicating that it contained corpuscular or non-cubic crystals. The latter authors corroborated these results by experiments in which anisotropy of flow was demonstrated by allowing a goldfish to swim in a glass tray filled with a diluted virus suspension [11]. When the glass tray was placed between crossed Nicol prisms, flow birefringence was shown to occur as a result of parallel orientation of corpuscles, possibly rod-shaped particles, by the movement of the fish. Additional strong support for the hypothesis that TMV suspensions consisted of particles was provided by X-ray diffraction studies carried out by Bernal and Fankuchen. From their results they concluded that the TMV particles had a width of approximately 15 nm and a length of at least ten times the width. They could even deduce that each TMV particle consisted of identical, regularly arranged subunits [22].

Finally, the virus particles themselves could be visualized, thanks to the construction of the first electron microscope [108]. Electron microscope specimens of TMV suspensions showed rod-shaped particles, approximately 15 nm wide and 300 nm long. Ever since, various improvements in preparing specimens for examination have been achieved, thus providing routine methods for electron microscopy of viruses[14]. By applying special fixation and embedding techniques, infected tissues and cells could be studied with the help of a microtome, providing ultrathin sections[15]. After elucidation of the nature of TMV, a large number of viruses could be characterized by making use of improved purification techniques. The application of ultracentrifugation, in particular centrifugation in density gradients as developed by Brakke, has been of great significance [30].

The first confrontation with viruses: symptoms

Early records make mention of abnormalities in plants that are now known to be caused by viruses. In a poem, the Japanese empress Koken, who lived around 750 A.D., expressed her mood about a leaf yellowing which gave shrubs of *Eupatorium lindleyanum* a wintry appearance although it was summertime[16].

[14]For instance, [82].
[15]For instance, [33, 82].
[16][120: 1].

Another – famous – example concerns the colour breaking of tulip flowers consisting of a kind of variegation in originally plain-coloured plants. In the first half of the 17th century, when the tulip became popular in Holland, bulbs of plants with such variegated flowers (flamed or broken tulips) were valued highly. As far back as 1637, growers already knew how to transmit this condition by grafting bulbs with flamed flowers to those with self-coloured ones[17]. Another variegation occurring in *Abutilon striatum* was also found to be transmissible by grafting, as reported by Lemoine in 1869[18]. Around that year, plants of that species, imported from South America, became popular as an ornamental in Europe. Incidentally, a variegated plant was found among those imported specimens. An attempt to get both a green and a variegated plant on one rootstock failed, because the green one became variegated. Nearly 80 years later, it was shown that in South America the virus responsible for the disease is spread by a whitefly species [133].

Shortly after tobacco mosaic was recognized as a virus disease, the virus aetiology of many other plant diseases was established. Soon it became clear that virus-diseased plants exhibit a gamut of symptoms, ranging from mosaic and ringspot patterns to necroses of leaves, stems, roots, bulbs and tubers, and often retardation of growth, dwarfing and stunting.

In the beginning, there was a tendency to consider "mosaic" in different crops to be caused by the same virus. But experimental transmission, in which sap from mosaic-diseased plants was introduced into a variety of healthy species and/or cultivars, revealed that, in general, there were great differences between mosaic-causing viruses. In this connection a few terms have to be explained. Deliberate introduction of a virus into a plant is called inoculation. The above-mentioned set of healthy test plants is commonly used to establish a so-called host range of the virus, i.e., those plant species and/or cultivars that get infected by the virus (susceptible plants). Plants that do not get infected are insusceptible or immune. A susceptible plant may or may not show symptoms. In the former case the plant is called sensitive to the virus; in the latter case the plant is insensitive (tolerant) and the infection is latent. Sensitive plants are usually systemically infected, i.e., the virus has moved from the site of inoculation to other parts of the plant via the vascular system. If the symptoms are restricted to the site of inoculation, the plant is locally infected. A well-known example of local symptoms are the necrotic lesions appearing on *Nicotiana glutinosa* leaves upon inoculation with TMV [90].

Different genotypes of a plant species may vary both in susceptibility and in sensitivity, as host range studies have revealed. This variation provided the key to breeding for resistance in crops. From 1940 onwards, host range studies have become common practice. Price and Holmes used host ranges for characterization of viruses [141, 91]. To establish that the virus in the source plant was a single one and not a mixture, back-inoculations were performed from each plant in the

[17] [195].

[18] Reported in a letter by Lemoine addressed to Duchartre, Société impériale et centrale d'horticulture de France, cited in Journal II, 3 (1869), p 47 of this society.

test series to healthy plants belonging to the same species/cultivar as the source plant. In this way, symptomlessly infected plants could also be detected.

Host range studies were, and are still, useful to select a suitable species/cultivar that may act as an indicator plant for a given virus, i.e., a plant showing characteristic symptoms. Investigations on viruses of trees and shrubs are facilitated if herbaceous test plants are available [89]. Environmental conditions may play an important role in symptom expression. At temperatures below 30 °C, TMV incites local necrotic lesions on *N. glutinosa* leaves. Above 30 °C no necrotic lesions will develop, but only a systemic mosaic [154]. In tobacco (*N. tabacum*) no typical mosaic symptoms develop at 36 °C [99]. At that temperature, the plants are still susceptible, but they have lost their sensitivity.

Besides temperature, light may also affect symptoms. Baur was able to cure *Abutilon striatum* from mosaic by shading the infected plants and removing the symptomatic leaves [7]. However, a reduction of light intensity in summer to one-third increased the susceptibility to infection with the viruses of tobacco necrosis, tomato bushy stunt, tobacco mosaic and tomato aucuba mosaic [12]. The virus content of systemically infected leaves increased by reduced light in the case of the last-mentioned three viruses.

Susceptibility and sensitivity are also affected by other factors. It was found that the better the nutritional conditions for plant growth the higher the susceptibility of *N. glutinosa* leaves to TMV [8]. Besides externally visible symptoms, most virus-infected plants also show internal aberrations. Ivanovskij already mentioned the occurrence of peculiar inclusion bodies in cells of the light green areas of tobacco leaves infected with TMV [95]. Goldstein distinguished two types of cell inclusions: one of a hexagonal-crystalline shape and the other a noncrystalline, amorphous, amoeboid body [78]. She coined the term X-bodies for the latter, and she considered the crystals to be host reaction products. Later, they were shown to consist of regularly arranged TMV particles [173]. The amorphous bodies, on the other hand, contained both virus particles and other materials. Inclusion bodies have been shown to be of diagnostic value as they are characteristic of the virus or virus group. An extensive treatise on inclusion bodies and another paper on the subject have been presented by Christie and Edwardson [48, 49].

Quanjer was one of the pioneers who studied histological effects of a virus infection. In his search for the cause of a potato disease called leaf roll, he examined sections of diseased potato stems and noticed that phloem tissue had become necrotic. According to him, this necrosis was responsible for the accumulation of starch observed in leaves of affected potato plants [143]. Later, Murphy disclosed that starch accumulation preceded phloem necrosis [129]. However, Thung found that both starch accumulation and phloem necrosis were the result of inhibited movement of carbohydrates in the phloem [181]. Independently, several German authors suggested that this is due to the abnormal deposition of callose, a polysaccharide present in the phloem. The so-called Igel Lange test, based on staining callose with resorcin blue, was at one time widely used to discriminate between healthy and infected plants in potato certification schemes in vogue in various European countries [54]. Smith has reviewed plant virus diseases and their

symptoms [163]. Bos has streamlined the terms used for describing symptoms for greater uniformity [27]. Van Loon has presented an extensive review of various aspects of disease induction including alterations in plant metabolism [193].

Virus strains

Since 1924, a number of reports indicate that variants of viruses exist in nature. The existence of such variants was first established when beet curly top virus was passed through different hosts [42]. After passage through certain hosts, the virus caused much milder symptoms in beet as compared to the original isolate; the virus seemed to be attenuated [41]. Such attenuation was also observed in TMV when tobacco plants infected with the normal virus were grown for at least ten days at a temperature of approximately 36 °C. Under these conditions, the symptoms were much milder than those caused by the normal virus, and the virus isolated from the heated plants produced mild symptoms in plants grown under normal conditions [100]. These phenomena may be explained by assuming that the virus particles present in an infected plant are not uniform but, in fact, represent a population of variants, generally designated as strains. Which strain will dominate depends on its concentration in the inoculum and on selection pressure, either by the host or by environmental conditions.

Isolation of strains is facilitated when a local-lesion host of the virus is available. *Nicotiana glutinosa* or the hybrid *N. glutinosa* × *N. tabacum*, both reacting to TMV with local lesions, have been used for this purpose. Based on the assumption that one local lesion results from infection by one virus particle, each lesion was cut out and used as inoculum for a tobacco plant. In this way, single-lesion pure-line strains were obtained. Specific hosts have also been used to isolate strains. Sea-holly (*Eryngium aquaticum*), for instance, proved to be susceptible only to mild strains of TMV, whereas tomato was exclusively infected by severe strains [102].

A given isolate of a pure strain may, however, change by mutation. This has been proved by Jensen who occasionally observed bright yellow spots in addition to the common mosaic on leaves of tobacco infected with a single-lesion pure-line strain of TMV [97]. Inoculum prepared from such a spot produced yellow mosaic in tobacco plants. Price has presented further evidence for mutation [139]. Using cowpea (*Vigna sinensis*, now *V. unguiculata*) as a local-lesion host to obtain pure-line strains of cucumber mosaic virus, after many serial passages he isolated a strain that produced aberrant yellow spots in tobacco.

Methods to induce mutant strains have been developed by subjecting virus-infected plants or inocula to different treatments, such as irradiation with X-rays or the application of mutagenic chemicals, viz. nitrogen mustard [109] or nitrous acid [111, 128]. Symptoms, though important, are not the only criteria used to distinguish between virus strains. They may also be differentiated by vector relationships [24] or by the amino acid composition of their coat protein [110].

Two strains infecting the same plant may exchange their genomic material when both are multiplying in the same cell. This, however, is very difficult to prove

experimentally. Recombination experiments have been successful with multicomponent (multiparticle) viruses possessing different nucleoprotein particles (see section on how to differentiate between viruses). Exchange of particles between two strains of such viruses led to the formation of new strains, so-called pseudo-recombinants [74, p 163]. True recombinants, on the other hand, are those whose genome consists of nucleotide sequences derived from both strains, assembled into one strand.

Interference between virus strains

An inhibitory effect of one virus strain on the other has been described by Thung, who reported that tobacco plants inoculated with a green strain of TMV produced no further symptoms if subsequently challenge-inoculated with a yellow strain [182, 183]. Similar phenomena have been described for other plant-virus combinations [153, 36, 140]. The term cross-protection has been introduced for this type of antagonism between virus strains; several theories have been launched to explain it, but its mechanism is not yet understood. Most likely, several mechanisms are involved, and there are indications that the host plant plays an important role.

Synergism of unrelated viruses

A plant infected by a combination of two unrelated viruses may show symptoms which differ from those induced by each one separately. This was first demonstrated by Bennett, who studied the effect of some viruses on plants infected with dodder latent mosaic virus (DLMV) [20]. This virus has a large host range. It incites marked symptoms in certain species, but the plants gradually recover and the virus concentration decreases to a low level. When tomato plants recovered from DLMV were inoculated with TMV, symptoms of DLMV not only reappeared, but the plants became, and remained, dwarfed, a condition typical for such doubly infected tomato plants. Moreover, an increase in the concentration of DLMV was noticed.

Later it was found that the concentration of potato virus X in tobacco plants doubly infected with potato virus Y attained a much higher level than in plants infected with potato virus X alone. The symptoms in the doubly infected plants were much more severe. On the other hand, the concentration of potato virus Y was not affected. The increase in the concentration of potato virus X, like that of DLMV in Bennett's experiments, seems to determine the severity of the synergistic reaction.

Genomic masking and transcapsidation

Another strain-related phenomenon is called genomic masking. It has been discovered in a tobacco plant mixedly infected with the normal (type) strain of TMV and an unstable mutant of this virus [3]. The latter produces defective coat protein unfit for assembly with the TMV-RNA. In the mixedly infected tobacco plant, virus particles were formed that contained either type TMV-RNA or mutant TMV-RNA. This was established by inoculation of *N. glutinosa* leaves with sap from the

doubly infected leaves, followed by testing of the lesions that had developed. Part of the lesions proved to contain the unstable mutant, showing that heterologous coating of its RNA with coat protein of the type strain had taken place.

Heterologous coating (transcapsidation) has also been observed in barley yellow dwarf virus [151]. Each of two strains of this virus, RPV and MAV, which are serologically unrelated, was found to have its own specific aphid-vector, viz., *Rhopalosiphum padi* and *Macrosiphum avenae*, respectively. When RPV and MAV were present in an oat plant, both strains could be transmitted by *R. padi*, i.e., also MAV. The explanation is that in the doubly infected plant, heterologous coating of MAV-RNA by RPV capsid protein had occurred, thus making transmission by *R. padi* possible. See also [65].

How to differentiate between viruses

In the absence of knowledge about the exact nature of a virus, most research in the first three decades of the 20th century was focused on its biological property, i.e., its infectivity. Virus literature in that period abounds with descriptions of diseases, including both macroscopic and microscopic symptoms, host ranges, transmission characteristics and infectivity of virus in crude sap. While studying host ranges, Holmes observed that some *Nicotiana* species produced local necrotic lesions upon inoculation of the leaves with TMV-containing tobacco sap [90]. Thanks to this discovery, the virus could henceforth be studied in quantitative assays, as numbers of local lesions were correlated to the virus concentration of the inoculum. Moreover, as pointed out in the section on virus strains, the property of certain viruses to incite local lesions on certain plant species was helpful in selecting strains.

As more and more virus diseases were being described, host ranges and symptomatology were soon found to be inadequate means for proper differentiation due to the fact that symptoms were very variable and depended heavily on the physiological condition of the plants and the virus source. Therefore, properties of a sap-transmissible virus in crude sap were considered to be more reliable for distinction of the different viruses. To establish these properties, three different tests were developed, viz. determination of the dilution end-point, thermal inactivation point, and longevity *in vitro* [101]. In the first test, the dilution end-point, defined as the highest dilution of sap from a virus-infected plant that was still infectious, was determined by inoculating each dilution on a number of assay plants, preferably local-lesion hosts. This test gave some information on the concentration of virus in the source plant. The thermal inactivation point was defined as the lowest temperature required for complete inactivation of a virus in crude sap heated for 10 min. Usually, the virus-containing sap was exposed to temperatures at 10 °C intervals. The heated suspensions were then inoculated on a number of assay plants. In longevity *in vitro*, the length of time was determined after which crude sap from a virus-infected plant lost its infectivity when kept at a temperature of 20–22 °C. Samples of crude sap were removed from storage at intervals and tested on assay plants. By these tests, only viruses that were either

very stable or very unstable could be characterized to some extent. TMV, for instance, was an example of a very stable virus with a thermal inactivation point of approximately 90 °C. Moreover, the sap was still infective at a dilution of 10^6 and after many years of storage. The virus of spotted wilt of tomato, on the other hand, already lost its infectivity between 40 and 46 °C and within approximately 2 h at 20–22 °C. But most viruses have thermal inactivation points between 55 and 70 °C.

However, even dilution end-point, thermal inactivation point and longevity *in vitro* were found to be of little diagnostic value, as they were also based on infectivity of the virus and as such subjected to the same conditions as host ranges and symptomatology. Another limitation of these tests was that they were applicable only to sap-transmissible viruses.

Even after the disclosure of the particle character of TMV, most of the research on plant viruses was still concerned with their infectivity. It was only after improvement of the resolution of the electron microscope and the development of electron microscopical techniques for the enhancement of contrast between virus particles and the supporting membrane on the grid, that progress was made in differentiation between viruses on the basis of their overall structure. The first of these techniques made use of shadow-casting, i.e., exposing virus particles on the grid to vapours of heavy metals, e.g., gold or palladium, resulting in contrast between areas where the electrons could pass and others where they could not [127, 208]. An even greater improvement was the introduction of the negative-staining technique in which the virus suspension was mixed with a solution of an electron-dense stain, e.g., sodium phosphotungstate [32]. In this way, structural details of the virus particle could be distinguished.

Up to 1952, the discovery that TMV contains a small amount of RNA had little impact on the type of research carried out in that period. The lack of interest in this viral component could be explained by the fact that it constituted only a very small part of a virus particle, at least in TMV. The importance of RNA came to light when Markham and Smith isolated turnip yellow mosaic virus and noticed that the purified preparation contained two types of particles: one possessing both protein and nucleic acid (35%) and the other, identical in appearance, consisting of protein only [119]. The nucleoprotein particle was infectious, the protein particle was not. Besides TMV particles, Takahashi and Ishii isolated a macromolecular protein from TMV-infected tobacco plants. This protein showed serological relationship with TMV and aggregated at pH 5.3 to rod-shaped particles resembling those of TMV. Apparently, it represented virus coat protein produced in excess [174].

Soon it was found that the RNAs of different viruses differed in their base composition. However, it was only in 1952 that the infectivity of the nucleic acid part was unambiguously demonstrated by Hershey and Chase in their experiments with a bacterial virus [87]. Some years later, a similar role of the nucleic acid part of a plant virus (TMV) was shown more or less simultaneously by Fraenkel-Conrat and Williams and by Gierer and Schramm [68, 76]. By elucidating the role of viral nucleic acid it had by then become possible to link infectivity of virus particles to their physico-chemical (intrinsic) properties. In these studies, density gradient

centrifugation, a technique developed for isolation and characterization of viruses, became a great asset [29]. This technique was based on the fact that sedimentation of particles depends not only on their mass, morphology and density, but also on the density of the medium. By varying the density, a good separation of particles was obtained. Using this technique, Lister was able to distinguish between the two types of rod-shaped particles – long and short ones – present in a purified preparation of tobacco rattle virus [112]. Later he confirmed that the long particles were infective, but unable to produce coat protein, so that the newly formed viral RNA remained unprotected, which led to unstable entities. The short particles, on the other hand, were not infective, but they produced coat protein. Both types of particles together gave rise to stable infectious entities [113].

The existence of multicomponent (multiparticle) viruses has been pointed out in the section on virus strains. The meaning of these terms is that the virus genome is divided in, for instance, two or three species of RNA (bi- or tripartite genomes) packaged in different particles. Tobacco rattle virus is an example of such a virus; it has a bipartite genome. Other examples of multiparticle viruses are cowpea mosaic virus, with a bipartite genome [34, 191], and alfalfa mosaic virus, with a tripartite genome divided among three different types of particles, necessary for stable infections [197]. Application of density gradient centrifugation also led to the discovery of so-called satellite viruses. Kassanis was the first to recognize a satellite virus in a culture of tobacco necrosis virus [106]. This satellite is completely dependent on tobacco necrosis virus for its replication; it lacks the genetic information for replication and needs tobacco necrosis virus as a helper in this respect. On the other hand, it does possess the genetic information for the production of coat protein. Consequently, the satellite differs in antigenic properties from the helper. Since then, other satellite viruses with their helpers have been identified [156]. Even a satellite of TMV has been found; it only replicates with the assistance of TMV, but has polyhedral particles [61].

In addition to satellite viruses, satellite RNAs have been described to occur in association with a number of plant viruses. The first satellite RNA was found with cucumber mosaic virus. Kaper and Waterworth showed the dramatic effect this RNA has in increasing the virulence of cucumber mosaic virus by inciting a lethal necrosis in tomato [104]. A review of satellite RNAs and the implications of their existence in association with their helper viruses is to be found in a number of chapters in the book edited by Vogt and Jackson [202].

With the increasing use of serology and development of more sensitive serological tests, it became possible to establish relationships between a large number of viruses. In this connection it is worth mentioning that first attempts to use serological affinities of viruses for their classification were already made by Chester in the nineteen thirties [46, 47]. So far, only viruses possessing single-stranded RNA genomes have been mentioned in this review. Actually, they constitute the bulk of plant viruses. There are, however, a few viruses with double-stranded RNA, such as wound tumor virus [25], and double-stranded DNA, such as cauliflower mosaic virus [157]. Single-stranded DNA genomes are found in, among others, the geminiviruses [79].

More complex viruses are plant rhabdoviruses, with lettuce necrotic yellows virus and potato yellow dwarf virus as representatives. They are bacilliform, have a membrane, and contain a single-stranded RNA which is not infective; it is a so-called negative RNA strand. These viruses contain an RNA polymerase that directs the multiplication process in the infected cell [69, 134]. Another virus having a membrane is tomato spotted wilt virus. The spherical particle has a single-stranded RNA segmented into three parts. The whole genome is contained in one virus particle, hence it is a virus with a divided genome, but not a multiparticle virus [188]. By pooling data on the physico-chemical characteristics and serological properties of viruses, the foundation was laid for a classification of viruses instead of a grouping of virus diseases, based on symptoms and/or transmission characteristics, as has been done earlier. A first promising virus classification was made by Brandes and Wetter who based their system on the morphology of viruses and their serological affinities. Plant viruses sharing a number of intrinsic properties turned out to also have some other properties in common, such as, for instance, mode of transmission and properties in crude sap [31].

In order to develop an internationally accepted nomenclature and classification of viruses, an International Committee on Nomenclature of Viruses (ICNV), with a subcommittee on plant viruses, was set up in 1966. The name was later changed to International Committee on Taxonomy of Viruses (ICTV). It was decided to arrange plant viruses with many known characteristics into groups, not into families, as had been done for viruses of animals and bacteria (exceptions: the families *Reoviridae* and *Rhabdoviridae*, with members in both animal and plant viruses). Each group consisted of viruses with a number of similar characteristics, for instance the tobacco mosaic virus group, later called the tobamovirus (a code name or siglum) group, made up of viruses resembling TMV.

The use of so-called cryptograms in addition to the vernacular name of the virus has been proposed [75, 73]. Cryptograms consisted of multiple criteria, viz. four pairs of characters in coded form, such as the type and strandedness of the nucleic acid, relative molecular mass of the nucleic acid and percentage of nucleic acid in the particle, outline of the particle and of its nucleocapsid, and types of host and vector. However, as more and more properties became known, and extension of cryptograms was considered to make them unmanageable, the system was given up in 1978. Thereafter, for a number of years, the siglum name was incorporated into the virus name, e.g., tobacco mosaic tobamovirus. That practice has been officially abandoned, too, and presently, the vernacular name refers to the virus species, whereas the sigla are used for the names of genera (e.g., *Tobamovirus*) or, in case of viruses with genera classified into a higher taxon, for the names of families (e.g., *Potyviridae*).

Since the publication of the first complete nucleotide sequence of a plant virus RNA in 1980, more and more viruses have been sequenced and their gene functions established. This has led to a drastic revision of a number of taxa [194, 66].

Transmission of viruses

Before anything was known about viruses in plants, some incidental transmission had been accomplished. As pointed out in the section on symptoms, grafting was practised in the 17th century by tulip growers to transmit the broken-tulip condition and in the 19th century for the transmission of *Abutilon* mosaic. Mechanical transmission of TMV was performed by means of a capillary tube filled with sap from diseased tobacco plants [122].

Soon it became clear that the contagious agents differed in their transmissibility. Some proved to be readily transmitted with extracts from infected plants, while experimental transmission of others was only possible by grafting.

Successful sap transmission was shown to be dependent on a number of factors. No transmission occurred when the virus concentration in the inoculum was too low. Transmission was also hampered by the presence of substances in the inoculum that inhibited the infection process. Many woody plants and those belonging to the family Rosaceae, e.g., strawberry, contain tannins (phenolic compounds), which were found to irreversibly combine with the virus particles, thus making them noninfective [9]. As nicotine was known to bind tannins, it has been successfully used to prepare infectious virus extracts, for instance, those from dahlia infected with dahlia mosaic virus [52]. A similar effect was shown by hide powder, which had proved effective in preparing infective inoculum of cacao swollen shoot virus from cacao leaves [35].

Besides these phenolic compounds, which affect the virus itself, some plants have been found to contain certain proteins or other substances that interfere with infection by making the plant less susceptible. Such a type of inhibition has been found to occur when a virus in *Phytolacca decandra* (= *P. esculenta*) could not be transmitted to other plant species, such as tobacco [2]. Later it was shown that a glycoprotein in the extract of *Phytolacca* leaves prevented the transmission to tobacco, but not to *Phytolacca* [107].

Rawlins and Tompkins increased the effectivity of inoculation by dusting the leaves with fine carborundum powder prior to rubbing them gently with inoculum [146]. This method is still being used in the study of all kinds of sap-transmissible viruses. Inadvertent mechanical transmission may occur by farming practices. Mayer narrates that in the tobacco-growing region, a farmer was nicknamed "Jantje Bont" ("Johnny Motley") by his colleagues because of the frequent occurrence of mosaic in his crop [122]. It was soon recognized that TMV may retain its infectivity in cured tobacco leaves and their products, as well as in contaminated plant debris in the soil. Potato virus X can easily be spread by farm implements. Also clothes may become a source of contamination when the farmer walks through an infected field, and even rabbits and dogs may carry the virus on their fur [184]. Cutting large potato tubers into small parts before planting, a common agricultural practice, also proved an effective means of spreading the virus in the crop [117]. Many of these contaminations can be prevented by hygienic measures, such as roguing of infected plants, disinfection of tools, etc.

In nurseries of fruit trees and certain ornamentals, grafting or budding is an old established means to propagate cultivars vegetatively. In this way, viruses have been easily transmitted from scion or bud to rootstock, and *vice versa*. Already in 1888, Erwin F. Smith reported that growers of peach trees had known for years that the condition "yellows" – now known to be caused by a virus – was transmitted by budding. For experimental transmission, other methods of grafting have also been used [162]. Nattrass transmitted a virus of passion fruit by leaf grafts to other plant species. He cut the petiole side-wise like a scion and inserted it into a slit made in the bark of a recipient plant [131]. In checking potatoes for the presence of virus, tuber grafting (core grafting) has been practised for more than 75 years [130]. As grafting is usually only successful when an organic union occurs between scion or bud and rootstock, this method cannot be used for taxonomically unrelated plants. Virus transmission between plants that do not unite in grafting is possible by means of dodder (*Cuscuta* spp.), a parasitic plant [18, 98]. When dodder parasitizes on a diseased plant and a healthy plant simultaneously, the virus may move from the infected plant through the sieve elements of dodder stems to the healthy plant, leading to infection of the latter [50]. Among the first reports on virus transmission through seed are those referring to bean common mosaic virus [149] and cucumber mosaic virus [63]. The incidence of seed transmission was shown to depend on a number of factors, such as the genetic make-up of the plant and the moment the mother plant got infected. In a few cases, pollen has been found to transmit virus, e.g., bean common mosaic virus [148].

In nature, most viruses are transmitted by vectors, i.e., organisms capable of transferring a virus from one plant to another over short or longer distances. Insects are among the most important vectors. The first indication that leafhoppers are involved in virus transmission came from a publication on rice stunt disease from Japan[19]. Gradually, more reports appeared on transmission of disease incitants by leafhoppers. For a long time, the leafhopper-transmitted pathogens were considered to be viruses, as has been pointed out earlier. However, it was later discovered that a relatively large number of them were not viruses, but mycoplasma-like organisms, e.g., the causal agents of aster yellows and sandal spike [118, 147] and rickettsia-like organisms, e.g., the incitant of Pierce's disease of grapevine and dwarf of alfalfa [77].

Remarkably, rice stunt virus was found to multiply in its vector after it had fed on an infected rice plant. This type of transmission is called circulative-propagative (after acquisition, the virus circulates through the insect and multiplies in it). Virus-carrying females even produced virus-infected offspring. The virus was transmitted through the eggs in several successive generations, but no transmission was observed via males [70–72]. This implies an intimate relationship between virus and vector. One may now wonder whether rice stunt virus is a plant virus or

[19]Gibbs and Harrison mention the work of Japanese workers who, at the end of the 19th century, discovered the role of leafhoppers in the spread of a dwarfing disease in rice. At that time the virus nature of the incitant was not yet known [74: 6].

an insect virus! Not all leafhopper-transmitted viruses multiply in their vector; they merely circulate within the latter. Around 1910, the leafhopper *Eutettix tenellus* was thought to cause beet curly top [185, 5], but later research revealed that the insect acted merely as vector of beet curly top virus [6, 21]. During feeding on healthy plants, the virus content of the vector decreased progressively. Virus-free specimens of *E. tenellus* became viruliferous while feeding on a sugar solution containing an extract of infected beet plants or viruliferous *E. tenellus*. In this way, certain properties of the virus could be determined [17].

Many more viruses are known to be transmitted by aphids. Watson and Roberts distinguished two types of mechanisms in aphid transmission, viz., persistent and nonpersistent transmission [204]. Persistently transmitted viruses (circulative viruses) are acquired from a diseased plant during a long access period, and the vector cannot immediately transmit the virus, as the latter has to circulate within the body of the insect to finally reach the salivary system. The period between the acquisition of virus by the vector and the moment the vector is able to transmit the virus is called the latent period. Nonpersistently transmitted viruses can be acquired by the vector in a few seconds after access to a virus-infected plant. As there is no latent period, the vector can transmit the virus to a healthy plant immediately upon acquisition, but it soon loses its ability to do so. Potato leafroll virus (PLRV) is persistent in its vector, *Myzus persicae*, whereas potato virus Y is nonpersistent in this aphid. It is generally assumed that the latter is acquired by superficial penetration of the leaves. In case of PLRV, on the other hand, the aphid has to reach the phloem to acquire the virus in sufficient quantity. Nonviruliferous aphids have been made viruliferous by transferring hemolymph from a PLRV-carrying aphid by micro-injection [53, 86]. Endosymbiont bacteria of the vector have been shown to be involved in transmission of PLRV. These bacteria produce symbionin, which has a binding activity with PLRV [187]. It is now known that many viruses are transmitted by vectors with the help of virus-coded proteins [116]. Some viruses are dependent on other viruses for their transmission. Tobacco rosette, a complex disease, is caused by two viruses, viz., tobacco vein distorting virus and tobacco mottle virus. The latter can only be transmitted by aphids if the former is present in the plant, too [165, 166]. This phenomenon is another example of transcapsidation, as mentioned above. The virus of *Abutilon* mosaic is transmitted by a whitefly, *Bemisia tabaci* [133]. This species is known to be a vector of several viruses, e.g., tobacco leaf curl virus and tobacco yellow net virus [199]. An individual whitefly may carry three different viruses simultaneously [198]. Already in 1927, *Thrips tabaci* was reported as a vector of tomato spotted wilt virus. Also, other Thysanoptera were shown to transmit this virus, which is widespread in the world and has a large host range. Only larvae, in contrast to the adults, become infective when feeding on an infected plant. They remain infective after moulting, reaching the imaginal stage [4]. The virus multiplies in the thrips *Frankliniella occidentalis* [206].

The first virus found to be transmitted by a beetle was cowpea mosaic virus [161]. Later, a few more were discovered, among others, turnip yellow mosaic virus [119]. Beetles proved to transmit readily after feeding for a few minutes

on an infected plant. Virus transmission is thought to take place by regurgitation from the foregut while feeding. Among other insect vectors, mealy bugs should be mentioned, both from the historical point of view and because of their economic importance. They were found to be the vector of cacao swollen shoot virus, the causal agent of swollen shoot disease, which had affected most cacao plantations in West Africa and had led to a dramatic drop in the production of cacao beans [28, 138, 137, 136]. The vector transmits the virus from jungle trees to cacao and from cacao to cacao, making eradication of the disease virtually impossible.

In 1955, the first reports appeared on the role of mites as vectors of certain viruses, viz., peach mosaic virus [209], fig mosaic virus [67] and wheat streak mosaic virus [160]. Already for some time there was a suspicion that certain viruses, such as wheat soil-borne mosaic virus [123][20], tobacco rattle virus [26][21], grapevine fanleaf virus[22], lettuce big-vein virus [96] and tobacco necrosis virus [164], are transmitted through the soil, but initially, no vectors could be identified. It was in 1958 that a nematode, *Xiphinema index*, was shown to be the vector of grapevine fanleaf virus [88]. Soon thereafter, *Trichodorus pachydermus*, another nematode species, proved to be the vector of tobacco rattle virus [168].

Around 1960, another soil-inhabiting organism was found to be a vector of certain viruses. The fungus *Olpidium brassicae* proved to be the vector of lettuce big-vein virus and tobacco necrosis virus [178, 37]. In the soil, particles of tobacco necrosis virus are adsorbed to the surface of the uniflagellate zoospores of the fungus. After adsorption, when the flagellum is withdrawn, virus particles are taken into the encysting zoospore. When the encysted zoospore penetrates a root, the virus is released into the cytoplasm of the root epidermal cells [180, 179]. Lettuce big-vein virus is transmitted in a different way. The virus is acquired by *O. brassicae* during development of the fungus in cells of a lettuce plant infected with the virus. Virus particles are inside the cytoplasm of zoospores and are retained in the resting spores [38]. Studies on the transmission of plant viruses have provided clues to understanding the epidemiology of virus diseases, which is a prerequisite for effective virus control in crops.

Establishment of infection and translocation of virus within the plant

Plant viruses transmitted mechanically or with the help of vectors need wounds to enter a plant. Wounding is thought to break the cuticle, the wall of epidermal cells and, most probably, the plasmalemma, thus giving access to the cytoplasm. Most research on the infection process has been carried out with TMV. By analogy with

[20] McKinney originally called this disease rosette disease of wheat [123].

[21] Böning named this tobacco disease "Streifen- und Kräuselkrankheit" ("stripe end frizzle disease") [26]; later it became internationally known as rattle disease, and the virus as tobacco rattle virus.

[22] According to Hewitt and co-workers it was L. Petri who in 1918 showed a connection between grapevine fanleaf ("arricciamento della vita" in Italian) and the soil [88].

bacteriophages, the virus was supposed to adsorb to so-called "receptor sites" or "infectible sites" [207] at the surface of the cytoplasm. The adsorbed virus particles would then release their nucleic acid ("uncoating") prior to replication of the latter. Newly formed TMV-RNA and coat protein would then assemble to virus particles. The events occurring shortly after inoculation (the so-called early events) have been studied extensively.

Indications for the existence of "infectible sites" were obtained from experiments in which the virus and an inhibitor of infection (extracts of carnation leaves containing a low-molecular-weight protein) were shown to be competitive. This competition was thought to be for "receptor sites" [192]. Indirect evidence for the process of uncoating has been obtained in different ways. In one type of experiment, no infection occurred when tobacco leaves were infiltrated with ribonuclease shortly after inoculation with TMV. When, however, the ribonuclease treatment was given two hours after inoculation, the leaves got infected [43, 84]. The following explanation was given. Shortly after inoculation, uncoating had started, leading to the production of free TMV-RNA, which was sensitive to the ribonuclease. After two hours, the uncoated RNA had by then reached a site in the cytoplasm where it was protected from the action of the enzyme. Indications for an uncoating period of two hours were also obtained by stripping the epidermis of leaves at different intervals after inoculation. This method had already been used in the early nineteen thirties to establish the time when virus had moved from the epidermis into the underlying mesophyll [186]. In order to demonstrate the uncoating period, the epidermis of leaves of *Nicotiana glutinosa* was stripped at different intervals after inoculation with either TMV or TMV-RNA, followed by testing the stripped epidermis for its virus content. At a temperature of approximately 20 °C, virus increase was observed 14 h after inoculation with TMV, and already after 12 h when TMV-RNA had been used as inoculum. This difference of two hours also pointed to a period during which uncoating might have taken place [59, 60].

Movement of virus from cell to cell was considered to take place via plasmodesmata. There is an intriguing picture of an ultrathin section showing polyhedral particles of strawberry latent ringspot virus in a plasmodesma between two mesophyll cells [150]. Tubular structures formed in the infected cells play an important role in the movement of virus to neighbouring cells. Besides movement from cell to cell there is long-distance movement along pre-existing pathways that are likewise modified in the infection process [40]. Molecular techniques have allowed identification of viral genes involved in movement from cell to cell. The majority of plant viruses encode a so-called movement protein, i.e., a nonstructural protein required for short-distance movement. Besides this protein, in some cases the viral coat protein has also been shown to play a role in this type of movement. This has led to distinction of two types of movement strategies: coat protein-independent movement and coat protein-dependent movement [145].

In contrast to the relatively slow movement of virus from cell to cell, its long-distance spread is usually more rapid. Classic experiments were performed by Samuel, who studied the movement of TMV in tomato plants. He inoculated a

terminal leaflet of a leaf situated at about one-third of the stem length from the soil surface. On the third day, the virus had moved out of the leaflet, and on the fourth day it had already reached the roots. From then on it moved upward, and on the fifth day the upper leaves got infected [155]. Evidence that a virus like TMV moves over long distances in the phloem was provided from experiments showing that virus spread is affected by the flow of metabolites in the plant [19]. Remarkably, ultrathin sections of leaves of lettuce plants infected with lettuce necrotic yellows virus revealed the presence of virus in young xylem cells of the veins, but not in the phloem. Moreover, xylem sap from the stem proved to contain infectious virus [45]. Apical meristems of some systemically infected plants were shown to be free of virus [125]. Such uneven distribution of virus in a plant provided an opportunity to free virus-infected vegetatively propagated cultivars from virus by growing plants from isolated meristems. An important step forward was the isolation of protoplasts from plant cells to study the infection process. Takebe and co-workers were able to isolate intact mesophyll cells from tobacco leaves infected with TMV. They could keep them alive for at least 24 h. From such isolated cells they obtained infected protoplasts [177]. Cocking and Pojnar went further. They isolated protoplasts from uninfected tomato fruit cells, exposed them to TMV and studied the course of infection with the electron microscope [51]. Infection *in vitro* of tobacco mesophyll protoplasts by TMV was also achieved [176].

Viruses as molecular pathogens

The discovery that the nucleic acid part of the virus is responsible for the latter's infectivity gave rise to extensive research on the infection process, particularly the replication of nucleic acid. In the beginning, investigations on these aspects of plant viruses were lagging behind those carried out on viruses of animals and bacteria. The reason for this was the unsuitability of plant tissues for infection studies. In contrast to animal tissues and bacteria, it was impossible to get synchronous infection of plant tissue due to the rigid cell walls. Moreover, plant cells lacked the uniformity of bacteria. Nevertheless, some progress was made after it had been demonstrated that the single-stranded DNA of a bacteriophage turned into a double-stranded form in infected bacteria [159]. Such double-stranded virus-specific nucleic acid occurred in cultured tumour cells infected with a human single-stranded RNA virus [124]. It was called the replicative form (RF) of the viral nucleic acid, as it was considered to be essential for replication. Soon the RF was demonstrated in tobacco leaves infected with TMV [158]. As was to be expected, the RF of TMV and the RF of turnip yellow mosaic virus were resistant to ribonuclease [144]. Nucleotide analysis of the RF of TMV showed that it consisted of the parental type viral RNA strand and its complement [205]. After demonstration of the existence of messenger RNA and the discovery of the genetic code by Nirenberg and Matthaei in 1961 [132] – a turning point in the life sciences – replication was then thought to proceed as follows: First, viral RNA acts as a messenger for the production of an enzyme (replicase). With the help of this enzyme, a complementary RNA strand is synthesized, leading to RF formation.

The replicase then attaches itself to the complementary strand and copies it to produce RNA identical in its nucleotide sequences to the original virus RNA.

Although in broad outline this concept of replication is still valid, many questions remained, such as, for instance, how a virus enters a cell, how a virus gets rid of its protein coat, which part of the viral RNA acts as a messenger RNA, how the coat protein is formed and how its assembly with the newly formed viral RNA strands takes place. Gradually, many of these questions could be answered, as new techniques were developed to facilitate studies of the infection process. One such technique was the isolation of protoplasts, as mentioned in the previous section. In the period between 1973 and 1983 also a host of novel techniques for detection of proteins, nucleic acids and viruses became available. Their application in virus testing has been presented in the book edited by Jones and Torrance [103]. The techniques include gel electrophoresis, enzyme-linked immunosorbent assay (ELISA), combinations of electron microscopy and serology such as immunosorbent electron microscopy (1973), decoration of virus particles with antiserum and labelling of virus particles with colloidal gold, nucleic acid hybridization in 1975, and, last but not least, the polymerase chain reaction (PCR) in 1983 by which, at least in theory, even a single DNA molecule may be amplified to a detectable amount of DNA. These advanced detection techniques made it possible to identify viral products formed during the infection process. Among these products were the helper proteins involved in virus transmission by vectors [135] and the movement proteins responsible for transport of virus from cell to cell [145]. Isolation of restriction endonucleases, which cut large DNA molecules into shorter fragments, facilitated determination of the nucleotide sequences of nucleic acids and were essential for insertion of viral nucleic acid into a bacterial system (cloning). Recombinant DNA techniques, using complementary DNA (cDNA) hybridization followed by cloning, were already developed in 1972 for an animal virus, but it would take several years before it was applied to a plant virus: tobacco necrosis satellite virus [190, 189]. In this way, the complete nucleotide sequence of the RNA of this virus could be determined. Aided by the giant strides made in the area of information technology with its advanced computer analyses, many more plant viruses were characterized by their nucleotide sequences. The first infectious transcripts were obtained from cloned plant viral cDNA, and most research focused on the way viral genes are expressed, and on transcription and translation strategies of viruses [1].

In 1987, successful insertion of viral genes into plants was achieved by Bevan and co-workers, who used recombinant DNA technology [23]. The aim of these experiments was to engineer resistance in plants against viruses. In some cases, such transgenic plants indeed showed resistance to the virus whose gene had been incorporated into the genome of the plant [39]. It is now known that plants possess a natural defence mechanism against pathogens including RNA viruses: post transcriptional gene silencing (PTGS). In this RNA-mediated mechanism, a ds RNA is cleaved by a ds RNA-specific nuclease to produce fragments called small interfering RNA (siRNA). The siRNAs bind to a homologous mRNA transcript, which leads to degradation of the transcript by endonucleolytic cleavage and

silencing of the gene. Hence, when a viral RNA with a high homology to an endogenous gene is introduced into a plant, the transcript of both viral RNA and the endogenous plant gene are degraded. As was to be expected, viruses have developed a system to suppress PTGS. The helper component-proteinase, a non-structural protein of potyviruses, inhibits the formation of siRNAs in potyvirus-infected plants [203].

Control of virus diseases

For many years, breeding for resistance or tolerance has been – and still is – an activity to cope with plant diseases including those caused by viruses. Plant breeders test collections of cultivars, including the latter's native and wild relatives for their susceptibility and sensitivity to disease incitants, and thus select the specimens with the desired qualities. Such selected plants are then used for hybridization. Recombinant DNA techniques may eventually even lead to breeding of immune cultivars. The search for chemicals that might protect plants against virus infections (besides those meant to control virus vectors) or cure infected plants, has not been successful. Heat treatment, however, has yielded interesting results in some cases. Gibbs and Harrison[23] mention that Kobus in 1889 reported improvement of sugar cane ratoons suffering from sereh disease by immersing them in water at 50 °C for 30 min before planting. Kassanis freed potato tubers from potato leafroll virus by keeping them at 37.5 °C in a moist atmosphere for about 25 days. However, this treatment is less suitable for commercial use, because tubers deteriorate easily under these conditions [105]. Studies on plant virus ecology and epidemiology of plant virus disease have resulted in practical applications. Such studies included the identification of infection sources (both inside and outside the crops) and the ways viruses are spread. A number of these aspects have been dealt with in one of the preceding sections.

The strategy pursued in the production of seed-potatoes in various countries is a direct outcome of the results obtained from ecological and epidemiological studies[24]. In the first quarter of the 20th century, when it was recognised that viruses play a predominant role in potato culture, measures were taken to improve the quality of the planting stock. Certification schemes based on inspection and control were, therefore, developed with emphasis on freedom from disease and varietal purity. Regulations were, and are, frequently modified in the light of new developments in detection of virus and virus diagnosis. Monitoring aphid populations, especially the winged specimens, during the growing season has become common practice. Growing numbers of winged aphids increase the incidence of infection by aphid-borne viruses. A certain size of the population of winged aphids is a signal for lifting seed-potato tubers. After lifting, samples of tubers are examined in the laboratory, and standards for percentages of infected plants (in a field) and tubers (in a batch) are set for quality grades. A batch of seed-potatoes

[23]Gibbs and Harrison [74: 220].

[24]Various aspects are treated in: Viruses of potatoes and seed-potato production [55].

is certified only if it meets the standard of a grade. If not, it is to be used for direct consumption only. The principles of the production of prime seed and other planting materials apply to various other crops of herbaceous and woody plants as well.

Epilogue

It may have become clear that, gradually, the emphasis in plant virus research has shifted from viruses as disease agents (pathogens) to viruses as molecular entities. This trend can easily be explained in the light of development of advanced molecular-biological techniques, which have greatly facilitated studies of viruses *in vitro*. However, in spite of all knowledge of the viral genome, the actual mechanism by which virus induces a disease in a plant is still wrapped in darkness. A challenge for future plant virus research would, therefore, be to shed light upon the (molecular) interactions between virus and host that eventually result in disease of the plant.

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