



Review

Soilborne viruses: advances in virus movement, virus induced gene silencing, and engineered resistance

Jeanmarie Verchot-Lubicz*

Department of Entomology and Plant Pathology, Oklahoma State University, NRC 127, Stillwater, OK 74078, USA

Accepted 30 July 2002

Abstract

Until recently soilborne plant viruses were considered important only because they are causative agents for agricultural diseases. In recent years, soilborne plant viruses have played a significant role in advancing research into mechanisms of plasmodesmata transport, gene silencing, and engineered resistance to plant pathogens. Three different mechanisms by which viruses move through plasmodesmata have been identified using dianthoviruses, nepoviruses, and benyviruses. The infectious clone of the tobnavirus *Tobacco rattle virus* (TRV) has become an important tool for studying virus induced gene silencing and may be a tool to silence meristematic gene expression. Investigations of soilborne viruses may enable the development of new strategies for control of soilborne diseases. The potential use of pathogen-derived resistance to control soilborne viruses is currently being explored in several laboratories.

© 2003 Elsevier Science Ltd. All rights reserved.

Keywords: Soilborne viruses; Benyvirus; Dianthovirus; Tobnavirus; Furovirus; Pecluvirus; Nepovirus

1. Soilborne viruses: model systems for exploring virus-host interactions

The first suggestion that a virus may be soilborne came from a study describing a mosaic disease of wheat in 1925 [56]. Later the virus was designated as *Soilborne wheat mosaic virus* (SBWMV) [12]. Between the 1950s and 1970s virologists pursued the possibility that viruses could be soilborne and during those years nematode and fungal vectors of plant viruses were identified [1]. Within the last twenty years, 53 plant viruses belonging to 12 genera have been identified as soilborne viruses (Table 1) [15].

Table 1 provides a summary list of soilborne viruses that are either transmitted by nematodes or fungi. Less than 1% of all known plant-parasitic nematodes are viral vectors. The nematode vectors belong to *Longidorus*, *Xiphinema*, and *Trichodorus* spp. and are known to transmit dianthoviruses, nepoviruses and tobnaviruses (Table 1) [3]. Nematode transmitted viruses cause significant problems worldwide in fruit and vegetable crops. For example, the nematode-transmitted *Tobacco rattle virus* (TRV), is the causative agent of potato spraing disease and is a significant

problem throughout Europe and North America [18]. TRV causes necrotic areas on potato tubers, which can severely affect the marketability of a crop [3].

Fungi known to transmit viruses belong to the order Plasmodiophorales or Chytridiales (Table 1). They are all zoosporic parasites of algae and plant roots. Most species of the order Plasmodiophorales and Chytridiales are distributed worldwide but are not agronomically important. There are approximately 35 species belonging to the order Plasmodiophorales and only three species, *Polymyxa betae*, *Polymyxa graminis*, and *Spongospora subterranean*, are viral vectors. *P. betae* transmits *Beet necrotic yellow vein virus* (BNYVV), which are the causal agent of sugar beet rhizomania (Table 1). *Polymyxa graminis* is the vector for several viruses that cause diseases in cereals and peanut [11, 32]. *Spongospora subterranea* is the agent for powdery scab of potato and is the vector for *Potato mop top virus* (PMTV) [1,2,13]. The phylum Chytridiomycetes is quite large, containing over 500 species. However, only *Olpidium brassicae*, *Olpidium cucurbitacearum*, and *Olpidium radiale* transmit plant viruses. *Olpidium brassicae* transmits the varicosavirus *Lettuce big vein virus* (LBVV) which causes vein yellowing and can have a significant impact on the marketability of lettuce. Many of the viruses transmitted by *Olpidium* spp., such as *Cucumber necrosis virus*, *Melon*

* Tel.: +1-405-744-7895; fax: +1-405-744-6039.

E-mail address: verchot@okway.okstate.edu (J. Verchot-Lubicz).

Table 1
Viruses and their soilborne vectors

Vector	Virus genera	Proportion of viruses in each genus with known vectors ^a
Nematodes		
<i>Longidorus</i> ssp	<i>Nepovirus</i> ^b	5/27
<i>Xiphinema</i> spp.	<i>Nepovirus</i> ^b	7/27
<i>Longidorus</i> + <i>Xiphinema</i> spp.	<i>Dianthovirus</i> ^c	1/3
	<i>Nepovirus</i> ^b	1/27
<i>Trichodorus</i> spp.	<i>Tobravirus</i>	2/3
Plasmodiophorales:		
<i>Polymyxa</i> spp.	<i>Furovirus</i>	5/5
	<i>Benyvirus</i>	1/1
	<i>Pecluvirus</i>	1/1
	<i>Bymovirus</i>	6/6
<i>Spongospora</i> spp.	<i>Pomovirus</i>	1/1
Chytridiales:		
<i>Olpidium</i> spp.	<i>Necrovirus</i>	1/1
	<i>Varicosavirus</i>	3/3
	<i>Tombusvirus</i>	1/12
	<i>Dianthovirus</i> ^c	1/3

This list was compiled from information provided by the VIDE database [15] and from the 6th ICTV report [52] which provided updated information about the taxonomic classification of viruses with fungal vectors.

^a For some genera there are individuals that are nematode or fungus transmitted. Furthermore there are other individuals whose mechanism of transmission is either not known or that are not transmitted by nematodes or fungi. The proportions of viruses that are known to be either nematode or fungal transmitted per total viruses in each genus are indicated.

^b There are a total of 27 nepoviruses. *Longidorus* spp. and *Xiphinema* spp. collectively transmit 13 species of nepoviruses. *Peach rosette mosaic virus* is the only nepovirus transmitted both by *Longidorus* and *Xiphinema* spp.

^c There are three known dianthoviruses. *Sweet clover necrotic mosaic virus* is mechanically transmitted, *Carnation ringspot virus* is transmitted by both *Longidorus* and *Xiphinema* spp., and RCNMV is transmitted by *Olpidium radiale*.

necrosis virus, *Red clover necrotic mosaic virus* and *Tobacco necrosis virus*, cause necrotic diseases that are agronomically important [16,19,20] (Table 1).

Until recently our ability to explore soilborne-virus host interactions was limited by the lack of available research tools. Within the last fifteen years, however, a number of these problems have been overcome and significant advances have been made in the study of soilborne viruses at the molecular level. A very important step has been the development of infectious clones. Plasmids containing viral cDNAs have been transcribed to produce infectious viral RNA that can be inoculated to plants. Mutational analysis of these cDNA clones can be used to study viral protein functions. The second important development is the use of the green fluorescent protein (GFP) as a visual marker for virus infection. The GFP gene has been incorporated into many infectious clones and has been crucial for studying viral cell-to-cell movement, vascular movement, and gene silencing [49,68]. In fact, important contributions to our general knowledge of virus movement and gene silencing have been made with soilborne viruses. Also within the last

ten years, the first attempts to control soilborne viruses using pathogen-derived resistance have been made. Until now control of nematode- or fungus-transmitted viruses has been primarily through nematicides or fungicides. Genetically engineered resistance to nematode-transmitted viruses is potentially the best method to control soilborne viruses in the absence of chemicals.

The goal of this article is to explore significant discoveries in the areas of virus transport, gene silencing, and pathogen-derived resistance that have been made using soilborne viruses. The contributions of soilborne viruses to advancement of research in these three areas have not been highlighted before, although much of what we know about these three topics can be attributed to soilborne viruses. Within the last fifteen years there have also been significant advances made in the field of virus-vector interactions. Researchers are beginning to unravel the mechanisms governing nematode- or fungus-transmission of plant viruses. The topics of virus-vector interactions and virus replication have been reviewed elsewhere and will not be considered further here [14,31,33,45,55].

2. Nematode-transmitted viruses: cell-to-cell transport, recovery, and gene silencing

Nematode-transmitted viruses belong to three genera, *Nepovirus*, *Tobravirus*, and *Dianthovirus* (Table 1) [14,80]. Nepoviruses are bipartite RNA viruses that have a small protein linked to the 5' end (VPg) and a 3' poly (A) sequence (Fig. 1). Both genome segments encode large polyproteins that are proteolytically processed to produce mature proteins [84]. RNA1 encodes replication-associated proteins. RNA2 encodes one factor required for RNA2 replication, the viral movement protein, and the viral coat protein (CP) (Fig. 1). Cytological studies have revealed tubular structures in nepovirus infected cells [85]. These tubular structures are composed primarily of viral movement proteins and are hollow structures that extend between adjacent cells. Virion particles were detected inside isolated tubules and it is likely that the tubules serve as conduits for cell-to-cell movement of virion particles [85]. Tubule-transport systems have been described for viruses belonging to the genera *Nepovirus*, *Comovirus*, *Badnavirus*, *Tospovirus*, and *Caulimovirus* [35–38,85] and it is likely that their movement proteins are related [57]. Amino acid sequence and mutational analyses indicate that tubule-forming movement proteins contain an N-terminal domain that is responsible for polymerization of the tubules. The core and C-terminal domains are also conserved among these viruses but their functions are not known [57].

Tobraviruses are also positive strand RNA viruses and have a bipartite genome structure (Fig. 1). Each genome segment has a 5' methyl-guanosine cap and a 3' tRNA-like structure. RNA1 encodes two proteins required for viral replication, a single movement protein and a seed

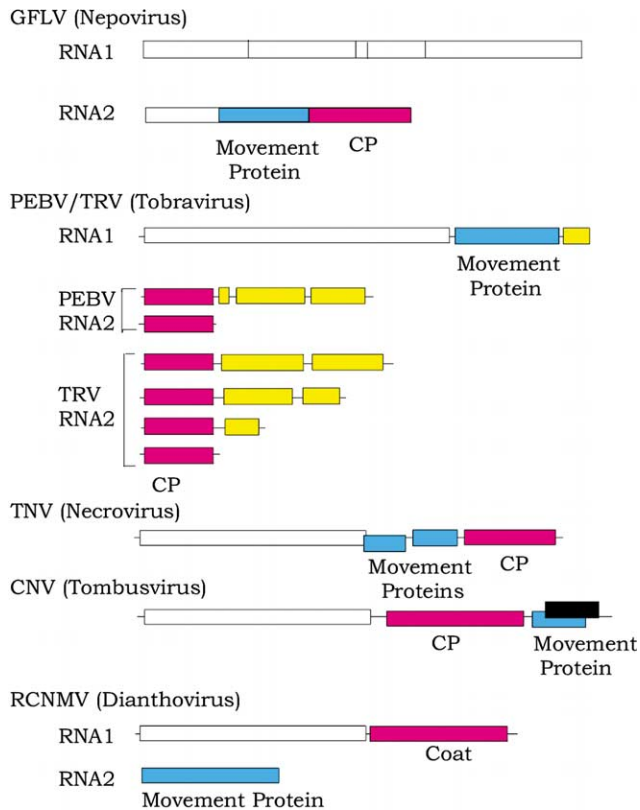


Fig. 1. Genome organization for six viruses transmitted either by nematodes or *Olpidium spp.* The open boxes indicate the open reading frames that encode proteins required for viral replication. Replicases may have multiple functions in virus replication including methyl guanosine transferase, polymerase, and helicase activities. Blue boxes indicate the coding sequences for each viral movement protein and red boxes indicate Coat Protein (CP) coat protein coding sequences. The yellow boxes indicate seed, nematode, or fungal transmission factors. The black box indicates an open reading frame that either regulates symptom expression, host range, or whose functions are not known. PEBV and TRV RNA2 segments that characterize different virus strains are indicated. The nematode transmission factors in different strains of PEBV and TRV vary in the number and molecular mass [58,59].

transmission factor [48,82]. The viral replicase is phylogenetically related to the tobamovirus replicase [39,40,80]. The seed-transmission factor is a cysteine-rich protein ranging from 12 to 16-kDa in size and is derived from the 3' region of the viral genome [48]. RNA2 encodes CP and nematode transmission factors [51]. Tobraviruses differ from other plant viruses in that the size of RNA2 varies among different tobiraviruses and even among related virus strains. Examples of known RNA2 segments that have been isolated from plants infected with *Pea early browning virus* (PEBV) or TRV are presented in Fig. 1. The coat protein gene is maintained in each RNA2. However, the numbers and sizes of the nematode transmission factors vary (Fig. 1). The variations in RNA2 have made it difficult to develop a unifying model explaining the requirements for nematode transmission of tobiraviruses.

The mechanism for tobiravirus cell-to-cell movement is likely to be distinct from the mechanism used by

nepoviruses. In phylogenetic comparisons of viral movement proteins, the tobiravirus 29-kDa movement protein is most closely related to the tobamovirus 30-kDa movement protein [34,57] and, therefore, it has been assumed that tobiraviruses and tobamoviruses share a common mechanism for cell-to-cell movement [17]. There has been very little research exploring the mechanism for tobiravirus movement but because of the close relationship between tobiraviruses and tobamoviruses a model describing the mechanism for tobamovirus cell-to-cell movement has also been used to describe the mechanism for tobiravirus cell-to-cell movement. In this model, viruses move from cell to cell in the absence of CP. Viral movement proteins cooperatively bind viral nucleic acids forming a ribonucleoprotein complex that is transported through plasmodesma into adjacent cells [17].

Tobiraviruses are unlike other bipartite plant viruses because they have the unique ability to cause two types of infections, known as NM (non-multiplying)- or M (multiplying)-type infections [45,80]. An NM-type infection is a local infection containing only RNA1. An M-type infection has encapsidated particles containing RNA1 and RNA2. RNA1 encodes the viral movement protein and, cell-to-cell movement of RNA1 can occur in the absence of RNA2 [45]. However, encapsidation is necessary for vascular movement, and only M-type infections can spread systemically. There may be mechanisms to ensure that RNA2 is maintained along with RNA1 as the virus spreads throughout the inoculated leaf. In addition, to its role in systemic spread of the virus in the plant, RNA2 is also required for nematode transmission.

Within the last decade the complete genome sequences for at least ten nepoviruses and three tobiraviruses have become available [15]. Infectious DNA clones of the nepovirus *Grapevine fanleaf virus* (GFLV) and the tobiraviruses TRV, PEBV and *Pepper ringspot virus* (PepRSV) have been made [48,49,50,79]. Mutations introduced into these infectious clones were used to identify factors necessary for virus replication, movement, seed transmission, and nematode transmission [8,82]. The GFP gene has been introduced into the TRV genome TRV.GFP to allow visualization of virus movement in inoculated plants [49]. The TRV.GFP infectious clone has also been adopted as a tool to explore virus accumulation in plant roots, nematode acquisition and gene silencing [49,65,68].

Plants inoculated with nepo- or tobiraviruses often recover from infection. In recovered plants, severe disease is often seen on inoculated and first systemic leaves while the upper leaves are symptom-free [67]. These upper leaves are resistant to secondary inoculation by the same virus [67]. The recovery phenomenon has been linked to homology dependent virus resistance and virus induced gene silencing VIGS [21,67,68]. A similar transgene induced 'recovery' phenomenon was reported in transgenic tobacco containing a nontranslatable version of the *Tobacco etch virus* (TEV) CP gene [21]. Inoculated leaves showed symptoms due to

TEV (a member of the *Potyvirus* genus), while the upper leaves were symptom-free [21]. The induced resistance in these plants was dependent on homology between the transgene and viral RNA. In the transgenic plants, as in the nepovirus-inoculated plants, recovered leaves were resistant to secondary inoculation with the homologous virus but were susceptible to infection by a heterologous virus [21,44,67].

VIGS is initiated in transgenic plants by a virus that shares nucleic acid sequence homology with the transgene [77]. The transgene RNA induces degradation of RNAs with similar sequence causing a reduction in the steady-state levels of transgene mRNAs and viral RNAs. This mechanism protects plants from systemic infection by RNA viruses and plants often appear to recover [81,83]. The mechanism for recovery seen in nepovirus- and tobnavirus-inoculated plants is likely to be similar to the mechanism described in transgenic plants [67,68,81]. Either nepo- or tobnaviruses contain sequence homology with the host genome thereby triggering VIGS, or the viruses use an alternative path for triggering the same RNA degradation mechanism.

Recovery and VIGS have been explored in studies using the TRV.GFP infectious clone [67,69]. GFP expression has been used to monitor virus accumulation in upper non-inoculated leaves. Fluorescence was initially detected in upper leaves but later GFP expression disappeared and the plants recovered from TRV infection. When TRV.GFP-recovered plants were inoculated with an unrelated virus they became infected [67]. When transcripts derived from an infectious clone of PVX which contained GFP coding sequence (PVX.GFP) were inoculated to the TRV.GFP-recovered plants, there was no evidence of PVX.GFP infection [68,69] indicating that TRV can trigger homology-dependent gene silencing VIGS.

The recovery phenomena demonstrated in nepo- and tobnavirus infected plants does suggest that gene silencing is a natural part of virus infection. Plant viruses may trigger an RNA-mediated defense mechanism that is manifested as a reduction in the steady-state levels of viral RNAs. In that case the ability of viruses to infect plants may require plant viruses to suppress this mechanism. Voinnet et al. (1999) used transgenic *N. benthamiana* plants expressing GFP to determine if plant viruses suppress gene silencing [81]. In this system *Agrobacterium tumefaciens* carrying a binary Ti plasmid vector that contains a 35S-GFP cassette was infiltrated into a mature leaf of a transgenic *N. benthamiana* plant. Within 20 days post inoculation GFP expression is shut off throughout the plant. A challenge virus was then used to inoculate the silenced plant. In silenced plants inoculated with the nepovirus *Tobacco black ringspot virus*, GFP expression was not restored. However, in silenced plants inoculated with TRV, gene silencing was suppressed and GFP expression was restored. The TRV gene product that suppresses gene silencing has not yet been characterized.

The TRV infectious clone has the potential to silence endogenous genes [68]. Since TRV is one of a few plant viruses that can enter meristematic cells, the TRV vector may be a tool for analyses of meristematic gene expression in plants [68].

3. Fungus-transmitted viruses: alternative models for viral transport and suppression of gene silencing

Known fungal vectors of plant viruses are zoosporic parasites of plant roots that belong to the orders *Chytridales* and *Plasmodiophorales* [6]. *Olpidium* spp., which belong to the order *Chytridales*, are the easiest to culture and, as such, have been useful for exploring the virus-vector interactions [1,6]. Viruses vectored by *Olpidium* spp. belong to the genera *Necrovirus*, *Tombusvirus*, *Dianthovirus*, and *Varicosavirus*. Surprisingly, of the three known viruses belonging to the genus *Dianthovirus*, one is nematode transmitted, one is transmitted by *O. radiale* and one is mechanically transmissible [15]. *Polymyxa* spp. and *S. subterranea* are plasmodiophorids and are vectors for viruses belonging to the genera *Furovirus*, *Benyvirus*, *Pecluvirus*, *Pomovirus*, and *Bymovirus* (Table 1) [1].

Viruses transmitted by plasmodiophorid vectors have been a source of controversy for taxonomists. These viruses were originally assigned either to the *Bymovirus* or *Furovirus* genus. In recent years, the genome sequences of many more viruses have been reported and at the last meeting of the ICTV (2000) viruses of the genus *Furovirus* were separated into four genera, *Furovirus*, *Pecluvirus*, *Benyvirus*, and *Pomovirus* [54,74]. The new classification takes into account variations in the numbers of genome segments, gene expression strategies and viral movement proteins (Fig. 2). Furoviruses represent a group of bipartite RNA viruses, an example of which is SBWMV [15]. The genus *Pecluvirus* contains bipartite RNA viruses related to *Peanut clump virus* (PCV). The genus *Pomovirus* contains tripartite viruses related to PMTV. Benyviruses resemble BNYVV and contain four to five genome segments [54].

Infectious clones of the *Cucumber necrosis virus* (CNV; a tombusvirus), *Red clover necrotic mosaic virus* (RCNMV; a dianthovirus), SBWMV, PCV, and BNYVV have been prepared. These infectious clones have been crucial for studies of virus movement and replication and have helped us to identify several new mechanisms for virus cell-to-cell and vascular transport. [30,66,87,89,91]. The combined results of mutational and phylogenetic analyses of these viral movement proteins indicate that there are at least two distinct classes of movement proteins encoded by these fungus-transmitted viruses [57].

The first class consists of tobamovirus-like movement proteins, which include those from tombusviruses, dianthoviruses, and furoviruses [57]. RCNMV, similar to the tobamovirus *Tobacco mosaic virus* (TMV), does not require the coat protein for cell-to-cell movement. The RCNMV

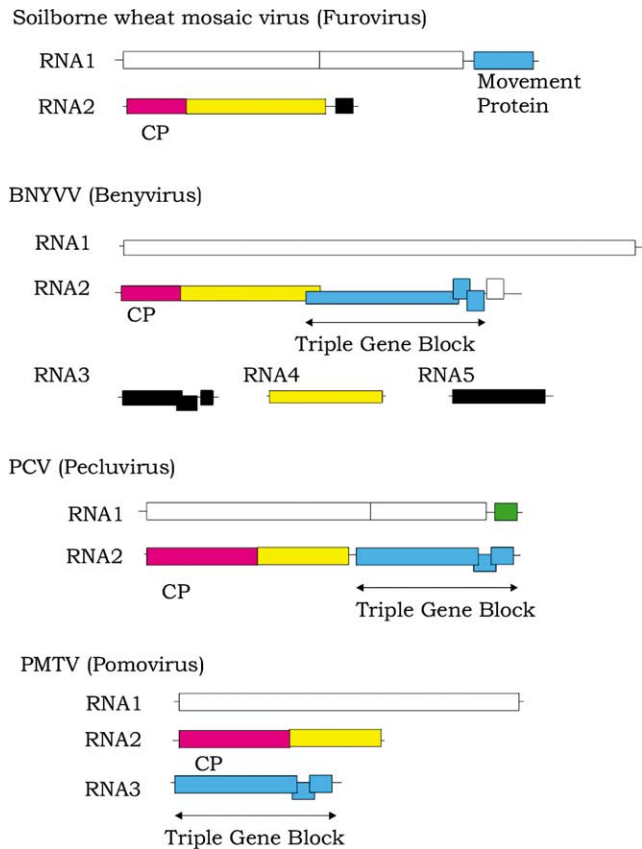


Fig. 2. Genome organization of four representative viruses transmitted either by *Polymyxa* spp. or *Spongospora subterranea*. Furoviruses and pecluviruses are bipartite RNA viruses, benyviruses have five genome segments and pomoviruses have three genome segments. Beny-, peclu-, and pomoviruses all have a triple gene block [24,30]. For each virus in this figure, translational readthrough of an amber stop codon located at the end of the CP open reading frame produces a large extension product. This readthrough domain of the CP, indicated in yellow, is required for fungal transmission of these viruses. The MP and CP coding sequences are indicated in blue and red as in Fig. 1. The green box indicates the P15 silencing suppressor of PCV.

movement protein has RNA binding activity, can induce expansion of plasmodesmata pores, and likely moves viral RNA through plasmodesmata [26,27,62,88].

The second class consists of the triple gene block proteins (named TGBp1, TGBp2, and TGBp3, respectively), which are found in beny-, pmo-, and pecluviruses (Fig. 2). The triple gene block is a genetic module that contains three overlapping open reading frames that are conserved among these soilborne viruses and among potexviruses, carlaviruses, and hordeiviruses [32,41,63]. Mutational analysis of the triple gene block proteins of BNYVV and PCV indicated that these proteins are essential for viral cell-to-cell movement [28,30]. TGBp1 of BNYVV and PCV have each been detected in association with plasmodesmata [24,25]. TGBp1 has RNA helicase activity and is translated from a monocistronic subgenomic RNA [10]. TGBp2 and TGBp3 are small membrane binding proteins and are derived from a bicistronic subgenomic

RNA [11]. Studies using BNYVV indicate that coordinated expression of TGBp2 and TGBp3 is important for viral cell-to-cell movement [11]. Replicons, which are modified viral RNAs that express foreign genes and are replicated by the virus, were used in studies of BNYVV cell-to-cell movement. Replicons expressing the individual triple gene block proteins or TGBp2 and TGBp3 together were used to complement BNYVV cell-to-cell movement defects *in trans*. Virus cell-to-cell movement could only be restored to defective viruses when TGBp2 and TGBp3 were provided from a bicistronic mRNA [11].

A model proposed for potexvirus cell-to-cell movement indicates that a ribonucleoprotein complex containing viral RNA, TGBp1, and CP move through plasmodesmata [46,47]. The TGBp2 and TGBp3 proteins function to promote plasmodesmata transport by anchoring the movement complex to the endoplasmic reticulum and then directing it toward the plasmodesmata [24]. PCV (a Pecluvirus) may differ from this model because CP is not required for viral cell-to-cell movement [30]. Thus in the case of PCV, CP is not likely to be part of the ribonucleoprotein movement complex.

The pathway for virus vascular movement from the roots to aerial parts of the plant has been explored in only a few studies. Evidence has been presented indicating the BNYVV, PMTV, and SBWMV are likely to use the xylem for vascular transport following natural inoculation of plants [22,78]. In immunogold labelling studies using light or transmission electron microscopy, BNYVV and SBWMV were each detected in xylem vessels or xylem parenchyma in infected plant roots [22,78]. SBWMV inclusion bodies were also identified in xylem parenchyma and mature xylem vessels in infected wheat roots [78].

Xylem transport by plant viruses was originally proposed in studies of sobemoviruses such as *Rice yellow mottle virus* (RYMV) and *Blueberry shoestring virus* [60,75]. The mechanism for xylem transport of sobemoviruses may be similar to that used by furoviruses. Like SBWMV, RYMV has been detected in immature xylem elements prior to programmed cell death. The virus is likely to move from cell to cell into immature xylem and then undergo replication. Then after programmed cell death, virus particles are released into the xylem and can move upward in the plant [60,78]. Since evidence of xylem transport was also found for sobemoviruses, it is likely that this model represents a general mechanism for xylem transport used by viruses that may or may not be soilborne.

Many fungal transmitted viruses that contain the triple gene block also contain a cysteine rich protein that, until recently, had no known function. Dunoyer et al. (2002) identified the PCV cysteine-rich P15 protein as a silencing suppressor [23]. The authors suggest that these cysteine rich proteins may be a class of silencing suppressors that exist primarily in fungal transmitted viruses [23]. This suggests that silencing suppression was acquired before these fungal transmitted viruses diverged.

4. Pathogen derived resistance as a strategy for control of soilborne viruses

Control of soilborne viruses in agricultural fields has relied primarily on using chemical nematicides or fungicides that reduce the vector populations but are not highly effective in limiting virus infection. Soil application of agrochemicals is now being restricted in many countries throughout Europe and the USA leaving farmers few alternatives to control soilborne viral diseases [53]. One option is to develop improved varieties by incorporating new sources of natural or engineered resistance into breeding programs. There are numerous cases in which transgenic plants expressing viral genes are resistant to viral infection. This is termed pathogen-derived resistance and has been employed in commercial varieties as a means of providing protection, primarily against viruses that are naturally inoculated to the leaves. Within the last decade studies have been conducted to determine whether pathogen-derived resistance could be used to control soilborne viruses.

Mechanisms of viral pathogen-derived resistance fall into two categories, i.e. protein-mediated or RNA-mediated. Protein-mediated resistance relies on the ability of the transgenically expressed viral protein to block essential steps in the virus infection cycle. Protein-mediated resistance was first reported in studies using transgenic tobacco expressing TMV CP. Transgenically expressed CP blocked virion disassembly in inoculated leaves, thereby preventing initiation of TMV infection [61,70,71,86]. There are also examples of replicase-mediated resistance and movement protein-mediated resistance in which mutant viral replicase or mutant movement protein genes expressed transgenically can block virus replication or cell-to-cell movement [7,43]. Transgenic RNA-mediated resistance is related to VIGS and as described earlier, was first demonstrated in transgenic tobacco containing TEV CP coding sequences. Accumulation of homologous viral- and transgene-derived RNAs triggers gene silencing thereby suppressing virus accumulation [29,44,72].

CP-mediated resistance has been tested in studies using nepoviruses or tobnaviruses and has not yet been tested against fungus-transmitted viruses. Transgenic plants expressing nepo- or tobnavirus CPs usually show a delay in infection but are not immune to virus infection. In general, CP-mediated resistance reduces systemic accumulation of viruses inoculated mechanically to the leaves. Few studies have assessed the effectiveness of CP-mediated resistance against viruses transmitted directly by nematodes. There have also been few attempts to engineer agronomically important crops for resistance to nepoviruses. GFLV and *Arabidopsis mosaic virus* (ArMV) cause devastating diseases to grapevine in Europe, and transgenic grapevine rootstocks containing the GFLV or ArMV CP genes have been made [41,73]. The resistance of these rootstocks is currently being tested.

Transgenic tobacco lines expressing CPs of four different nepoviruses, GFLV, *Strawberry latent ringspot virus* (SLRSV), *Grapevine chrome mosaic virus* (GCMV) or ArMV have been tested for resistance to the corresponding viruses. In studies using transgenic tobacco expressing GFLV CP, GCMV CP, or ArMV CP, virus was inoculated to the foliage. In each case virus accumulation was significantly reduced and systemic infection was often delayed [5,9,73]. The best example involved SLRSV CP transgenic tobacco plants that were resistant to systemic infection by SLRSV. There was no evidence of virus accumulation in upper non-inoculated leaves following either foliar inoculation of SLRSV or exposure of plant roots to viruliferous nematodes [42]. In the case of SLRSV, CP-mediated resistance may be useful in the field to block virus infection.

CP-expressing transgenic tobacco lines were tested for resistance to tobnaviruses following foliar inoculation of virus. Transgenic tobacco plants expressing the CP of TRV strain TCM or PLB displayed varying levels of resistance to different TRV strains [4,64,76]. While those expressing the CP of TRV-TCM were resistant to TRV-TCM but were susceptible to inoculation with TRV-PLB [76]. Transgenic tobacco plants expressing the CP of TRV-PLB were resistant to TRV-PLB, -PPK20, -PPW1 and -PPS1 but not TRV-TCM. TRV-PLB shares significant sequence homology with TRV-PPK20, -PPW1, and PPS1 but not TRV-TCM, suggesting that resistance may be homology-dependent [4,64]. In some experiments, TRV accumulation in CP-expressing transgenic tobacco was blocked following foliar inoculation of virus, but not following exposure to viruliferous nematodes [64]. Thus CP-mediated resistance may not be useful to limit TRV accumulation in field grown crops [64].

Replicase-mediated resistance and RNA-mediated resistance caused a significant reduction in tobnavirus accumulation in transgenic tobacco. Studies have been conducted using only foliar inoculated viruses and there is no data yet to indicate the effects of either of these two resistance mechanisms to nematode transmitted viruses. To test replicase-mediated resistance, a PEBV gene for a 54-kDa protein gene that is a fragment of the PEBV replicase was used to prepare transgenic tobacco [52]. These plants were resistant to low and high (1 mg/ml) levels of PEBV containing inoculum, to related PEBV isolates, and to *Broad bean yellow band virus*. However, they were not resistant to infection by two other tobnaviruses, PepRSV and TRV, suggesting that these PEBV 54-kDa protein-expressing plants have the potential for limited protection against unrelated viruses [52]. RNA-mediated resistance was effective against *Tomato Ringspot virus* (TomRSV) in transgenic *N. benthamiana* and *N. tabacum* containing sense and antisense CP genes. These plants had low levels of transgene RNA and were completely resistant to TomRSV [90].

In conclusion, engineered resistance may, represent a tool for controlling soilborne diseases in the field. In particular,

RNA-mediated resistance and replicase-mediated resistance provide the best protection to transgenic plants. However, additional research is needed to test pathogen-derived resistance to soilborne viruses in agronomically important crops and to assess resistance to natural infections.

5. The future

In the last decade, soilborne viruses have contributed several new mechanisms for virus cell-to-cell and vascular transport. Plasmodesmata-mediated transport of viruses and viral proteins requires modification of the plasmodesmata and involves a selective mechanism that controls which proteins move between adjacent cells. This suggests that there may be recognition factors in the cell interacting with the virus for plasmodesmata transport. Since plant viruses use several mechanisms to move between cells, we cannot preclude the possibility that there are a number of cellular factors that are recognized by different viral movement proteins to facilitate virus movement from cell to cell. Perhaps, in the next decade, host proteins contributing to plasmodesmata transport will be characterized.

The possibility that viruses use the xylem for long distance transport has been raised in studies of several soilborne viruses. Since xylem vessels are not connected to the parenchyma or mesophyll by plasmodesmata connects, new models must be described for xylem loading and unloading of plant viruses. There is evidence that viruses may enter immature xylem elements, which have plasmodesmata connections with parenchyma cells. Thus, virus xylem loading would be developmentally regulated. We do not yet know if xylem transport is an essential component of viral long distance movement.

In recent years, numerous soilborne viruses have been identified and many of their genomes have been sequenced. The next decade is likely to focus on identifying unique features of their genomes that may provide new insights into of virus infection cycles and that may also lead to novel methods of control. Since there are societal pressures to reduce the use of agrochemicals in Europe and the USA, more attention must be focused on developing novel control methods. Pathogen-derived resistance has been studied extensively as a means to control foliar inoculated viruses, but has not yet been fully explored as an approach to controlling soilborne viruses. We do not know yet how effective pathogen-derived resistance will be as a method to control soilborne viruses in the field. We may need to develop other approaches. Since we also live in an era of functional genomics studies research may focus more on identifying host genes involved in susceptibility and resistance. As we learn more about the molecular basis for pathogenicity, perhaps alternative control methods will be developed. The challenge ahead will be to develop new

technologies that farmers can use to reduce the effects of soilborne diseases and maintain crop yields.

Acknowledgements

This work was approved for publication by the director of the Oklahoma Agricultural Experiment Station and supported in part under the project H-2371.

References

- [1] Adams MJ. Transmission of plant viruses by fungi. *Annals of Applied Biology* 1991;118:479–92.
- [2] Adams MJ, Jacquier C. Infection of cereals and grasses by isolates of *Polymyxa graminis* (*Plasmodiophorales*). *Annals Applied Biology* 1994;125:53–60.
- [3] Agrios GN. *Plant Pathology*, 4th edn. New York: Academic Press; 1997.
- [4] Angenent GC, van den Ouweland JMW, Bol JF. Susceptibility to virus infection of transgenic tobacco plants expressing structural and nonstructural genes of *Tobacco rattle virus*. *Virology* 1990;175: 191–8.
- [5] Bardonnnet N, Hans F, Serghini MA, Pinck L. Protection against virus infection in tobacco plants expressing the coat protein of grapevine fanleaf nepovirus. *Plant Cell Reports* 1994;13:357–60.
- [6] Barr DJS. Zoospore plant Parasites as Fungal Vectors of viruses: Taxonomy and Life Cycles of Species Involved. In: Cooper JI, Asher MJC, editors. *Developments in Applied Biology: Viruses with Fungal Vectors*, 2. New York: Assoc. of Applied Biologists; 1988. p. 123–37.
- [7] Baulcombe D. Replicase-mediated resistance: a novel type of virus resistance in transgenic plants. *Trends in Microbiology* 1994;2: 60–3.
- [8] Belin C, Schmitt C, Gaire F, Walter B, Demangeat G, Pinck L. The nine C-terminal residues of Grapevine fanleaf nepovirus movement protein are critical for systemic virus spread. *Journal of General Virology* 1999;80:1347–56.
- [9] Bertioli DJ, Cooper JI, Edwards ML, Hawes WS. Arabis mosaic nepovirus coat protein in transgenic tobacco lessens disease severity and virus replication. *Annals Applied Biology* 1992;120:47–54.
- [10] Bleykasten C, Gilmer D, Guilley H, Richards KE, Jonard G. *Beet necrotic yellow vein virus* 42 kDa triple gene block protein binds nucleic acid in vitro. *Journal of General Virology* 1996;77:889–97.
- [11] Bleykasten-Grosshans C, Guilley H, Bouzoubaa S, Richards KE, Jonard G. Independent expression of the first two triple gene block proteins of *Beet necrotic yellow vein virus* complements virus defective in the corresponding gene but expression of the third protein inhibits viral cell-to-cell movement. *Molecular Plant-Microbe Interactions* 1997;10:240–6.
- [12] Brakke MK, Estes AP, Schuster ML. Transmission of *Soil-borne wheat mosaic virus*. *Phytopathology* 1964;55:79–86.
- [13] Braselton JP. Current status of the plasmodiophorids. *Critical Reviews in Microbiology* 1995;21:263–75.
- [14] Brown DJF, MacFarlane SA. Worms that transmit viruses. *Biologist* 2001;48:35–48.
- [15] Brunt AA, Crabtree K, Dallwitz MJ, Gibbs AJ, Watson L, Zurcher EJ., (Eds.) Version: 20th August 1996 onwards, posting date. *Plant Viruses Online: descriptions and Lists from the VIDE Database*. URL <http://biology.anu.edu.au/Groups/MES/vid/>
- [16] Cambell RN, Fry PR. The nature of associations between *Olpidium brassicae* and *Lettuce big-vein* and *Tobacco necrosis viruses*. *Virology* 1966;29:222–33.

- [17] Carrington JC, Kasschau KD, Mahajan SK, Schaad MC. Cell-to-cell and long-distance transport of viruses in plants. *The Plant Cell* 1996;8:1669–81.
- [18] Cooper JJ, Harrison BD. The role of weed hosts and the distribution and activity of vector nematodes in the ecology of tobacco rattle virus. *Annals Applied Biology* 1973;73:53–66.
- [19] Dias HF. The relationship between *Cucumber necrosis virus* and its vector, *Olpidium cucurbitacearum*. *Virology* 1970;42:204–11.
- [20] Dias HF. Transmission of *Cucumber necrosis virus* by *Olpidium cucurbitacearum* Barr and Dias. *Virology* 1970;40:828–39.
- [21] Dougherty WG, Lindbo JA, Smith HA, Parks TD, Swaney S, Proebsting WM. RNA-mediated virus resistance in transgenic plants: exploitation of a cellular pathway possibly involved in RNA degradation. *Molecular Plant-Microbe Interactions* 1994;7:544–52.
- [22] Dubois F, Sangwan RS, Sangwan-Norreel BS. *Spread of Beet necrotic yellow vein virus* in infected seedlings and plants of sugar beet (*Beta vulgaris*). *Protoplasma* 1994;179:72–82.
- [23] Dunoyer P, Pfeffer S, Fritsch C, Hemmer O, Voinnet O, Richard KE. Identification, subcellular localization and some properties of a cysteine-rich suppressor of gene silencing encoded by *Peanut clump virus*. *The Plant Journal* 2002;29:555–67.
- [24] Erhardt M, Morant M, Ritzenthaler C, Stussi-Garud C, Guilley H, Richards K, Jonard G, Bouzoubaa S, Gilmer D. P42 movement protein of *Beet necrotic yellow vein virus* is targeted by the movement proteins P13 and P15 to punctate bodies associated with plasmodesmata. *Molecular Plant-Microbe Interactions* 2000;13:520–8.
- [25] Erhardt M, Stussi-Garud C, Guilley H, Richards KE, Jonard G, Bouzoubaa S. The first triple gene block protein of *Peanut clump virus* localizes to the plasmodesmata during virus infection. *Virology* 1999;264:220–9.
- [26] Fujiwara T, Giesman-Cookmeyer D, Ding B, Lommel S, Lucas WJ. Cell-to-cell trafficking of macromolecules through plasmodesmata potentiated by the Red clover necrotic mosaic virus movement protein. *The Plant Cell* 1993;5:1783–94.
- [27] Geisman-Cookmeyer D, Lommel SA. Alanine scanning mutagenesis of a plant virus movement protein identifies three functional domains. *The Plant Cell* 1993;5:973–82.
- [28] Gilmer D, Bouzoubaa S, Hehn A, Guilley H, Richards K, Jonard G. Efficient cell-to-cell movement of *Beet necrotic yellow vein virus* requires 3' proximal genes located on RNA 2. *Virology* 1992;189:40–7.
- [29] Goodwin J, Chapman K, Parks TD, Wernsman EA, Dougherty WG. Genetic and biochemical dissection of transgenic RNA-mediated resistance. *The Plant Cell* 1996;8:95–105.
- [30] Herzog E, Hemmer O, Hauser S, Meyer G, Bouzoubaa S, Fritsch C. Identification of genes involved in replication and movement of *Peanut clump virus*. *Virology* 1998;248:312–22.
- [31] Hiruki C. Multiple transmission of plant viruses by *Olpidium brassicae*. *Canadian Journal of Plant Pathology* 1994;16:261–5.
- [32] Huisman MJ, Linthorst HJM, Bol JF, Cornelissen BJC. The complete nucleotide sequence of *Potato virus X* and its homologies at the amino acid level with various plus-stranded RNA viruses. *Journal of General Virology* 1988;69:1789–98.
- [33] Hull R. *Matthew's Plant Virology*, 4 ed. New York: Academic Press; 2002.
- [34] Hull R. The movement of viruses in plants. *Annual Review Phytopathology* 1989;27:213–40.
- [35] Kasteel D, Wellink J, Verver J, van Lent J, Goldbach R, van Kammen A. The involvement of *Cowpea mosaic virus* M RNA-encoded proteins in tubule formation. *Journal of General Virology* 1993;74:1721–4.
- [36] Kasteel DTJ, Perbal MC, Boyer JC, Wellink J, Goldbach RW, Maule AJ, van Lent JWM. The movement proteins of *Cowpea mosaic virus* and *Cauliflower mosaic virus* induce tubular structures in plant and insect cells. *Journal of General Virology* 1996;78:2089–93.
- [37] Kasteel DTJ, van der Wel NN, Jansen KA, Goldbach RW, van Lent JWM. Tubule-forming capacity of the movement proteins of *Alfalfa mosaic virus* and *Brome mosaic virus*. *Journal of General Virology* 1997;72:2197–206.
- [38] Kasteel DTJ, Wellink J, Goldbach RW, van Lent JWM. Isolation and characterization of tubular structures of *Cowpea mosaic virus*. *Journal of General Virology* 1997;78:3167–70.
- [39] Koonin EV. The phylogeny of RNA-dependent RNA polymerases of positive-strand RNA viruses. *Journal of General Virology* 1991;72:2197–206.
- [40] Koonin EV, Dolja VV. Evolution and taxonomy of positive-strand RNA viruses: implications of comparative analysis of amino acid sequences. *Critical Reviews in Biochemistry and Molecular Biology* 1993;28:375–430.
- [41] Krastanova S, Perrin M, Barbier P, Demangeat G, Cornuet P, Bardonnet N, Otten L, Pinck L, Walter B. Transformation of grapevine rootstocks with the coat protein gene of grapevine fanleaf nepovirus. *Plant Cell Reports* 1995;14:550–4.
- [42] Kreiah S, Edwards ML, Hawes WS, Jones AT, Brown DJF, McGavin WJ, Cooper JJ. Some coat protein constituents from *Strawberry latent ringspot virus* expressed in transgenic tobacco protect plants against systematic invasion following root inoculation by nematode vectors. *European Journal Plant Pathology* 1996;102:297–303.
- [43] Lapidot M, Gafny R, Ding B, Wolf S, Lucas WJ, Beachy RN. A dysfunctional movement protein of *Tobacco mosaic virus* that partially modifies the plasmodesmata and limits virus spread in transgenic plants. *Plant Journal* 1993;4:959–70.
- [44] Lindbo JA, Dougherty WG. Untranslatable transcripts of the *Tobacco etch virus* coat protein gene sequence can interfere with *Tobacco etch virus* replication in transgenic plants and protoplasts. *Virology* 1992;189:725–33.
- [45] Lister RM. *Tobacco rattle virus*. *Intervirology* 1986;26:61–73.
- [46] Lough TJ, Netzler NE, Emerson SJ, Sutherland P, Carr F, Beck DL, Lucas WJ, Forster RLS. Cell-to-cell movement of Potexviruses: Evidence for a ribonucleoprotein complex involving the coat protein and first triple gene block protein. *Molecular Plant-Microbe Interactions* 2000;13:962–74.
- [47] Lough TJ, Shash K, Xoconostle-Cazares B, Hofstra KR, Beck DL, Balmori E, Forster RLS, Lucas WJ. Molecular dissection of the mechanism by which potexvirus triple gene block proteins mediate cell-to-cell transport of infectious RNA. *Molecular Plant-Microbe Interactions* 1998;11:801–14.
- [48] MacFarlane SA. Molecular biology of the tobnaviruses. *Journal of General Virology* 1999;80:2799–807.
- [49] MacFarlane SA, Popovich AH. Efficient expression of foreign proteins in roots from *Tobravirus* vectors. *Virology* 2000;267:29–35.
- [50] MacFarlane SA, Gilmer D, Davies JW. Efficient inoculation with CaMV 35S promoter-driven DNA clones of the Tobnavirus PEBV. *Virology* 1992;187:829–31.
- [51] MacFarlane SA, Wallis CV, Brown DJF. Multiple virus genes involved in the nematode transmission of *Pea early browning virus*. *Virology* 1996;219:417–22.
- [52] MacFarlane SA, Davies JW. Plants transformed with a region of the 201-kilodalton replicase gene from *Pea early browning virus* RNA1 are resistant to virus infection. *Proceedings of the National Academy of Sciences USA* 1992;89:5829–33.
- [53] Mauro MC, Toutain S, Walter B, Pinck L, Otten L, Coutos-Thevenot P, Deloire A, Barbier P. High efficiency regeneration of grapevine plants transformed with the GFLV coat protein gene. *Plant Science* 1995;112:97–106.
- [54] Mayo MA. Developments in plant virus taxonomy since the publication of the 6th ICTV report. *Archives of Virology* 1999;144:1659–66.
- [55] MacFarlane SA. Molecular biology of the tobnaviruses. *Journal of General Virology* 1999;80:2799–807.
- [56] McKinney HH. A mosaic disease of winter wheat and winter rye. *United States Department of Agriculture Bulletin No.1361*. 1925
- [57] Melcher U. The 30 K superfamily of viral movement proteins. *Journal of General Virology* 2000;81:257–66.

- [58] Meulewaeter F, Cornelissen M, van Emmelo J. Subgenomic RNAs mediate expression of cistrons located internally on the genomic RNA of *Tobacco necrosis virus strain A*. *Journal of Virology* 1992;66: 6419–28.
- [59] Offei SK, Coffin RS, Coutts RHA. The Tobacco necrosis virus p7a protein is a nucleic acid-binding protein. *Journal of General Virology* 1995;76:1493–6.
- [60] Opalka N, Brugidou C, Bonneau C, Nicole M, Beachy RN, Yeager M, Fauquet C. Movement of *Rice yellow mottle virus* between xylem cells through pit membranes. *Proceedings of the National Academy of Science USA* 1998;95:3323–8.
- [61] Osbourn JK, Watts JW, Beachy RN, Wilson TMA. Evidence that nucleocapsid disassembly and a later step in virus replication are inhibited in transgenic tobacco protoplasts expressing TMV coat protein. *Virology* 1989;172:370–3.
- [62] Osman TAM, Hayes RJ, Buck KW. Cooperative binding of the *Red clover necrotic mosaic virus* movement protein to single-stranded nucleic acids. *Journal of General Virology* 1992;73:223–7.
- [63] Petty ITD, Jackson AO. Mutational analysis of *Barley stripe mosaic virus* RNA B. *Virology* 1990;179:712–8.
- [64] Ploeg AT, Mathis A, Bol JF, Brown DJF. Susceptibility of transgenic tobacco plants expressing *Tobacco rattle virus* coat protein to nematode-transmitted and mechanically inoculated *Tobacco rattle virus*. *Journal of General Virology* 1993;74:2709–15.
- [65] Prior DAM, MacFarlane SA, Oparka KJ, Brown DJF. Study of viral infection, and symplastic and systemic invasion of plants using green fluorescent protein tagged tobacco rattle tobavirus transmitted by *Paratrichodorus pachydermus* nematodes. *Russian Journal of Nematology* 1998;6:73.
- [66] Quillet L, Guilley H, Jonard G, Richards K. In vitro synthesis of biologically active *Beet necrotic yellow vein virus* RNA. *Virology* 1989;172:293–301.
- [67] Ratcliff F, Harrison BD, Baulcombe DC. A similarity between viral defense and gene silencing in plants. *Science* 1997;276:1558–60.
- [68] Ratcliff F, Martin-Hernandez AM, Baulcombe DC. *Tobacco rattle virus* as a vector for analysis of gene function by silencing. *Plant Journal* 2001;25:237–45.
- [69] Ratcliff FG, MacFarlane SA, Baulcombe DC. Gene silencing without DNA: RNA-mediated cross-protection between viruses. *The Plant Cell* 1999;11:1207–15.
- [70] Register JC, Beachy RN. Resistance to TMV results from interference with an early event in infection. *Virology* 1988;166:524–32.
- [71] Reimann-Philipp U, Beachy RN. Coat protein-mediated resistance in transgenic tobacco expressing the *Tobacco mosaic virus* coat protein from tissue-specific promoters. *Molecular Plant-Microbe Interactions* 1993;6:323–30.
- [72] Smith HA, Swaney SL, Parks TD, Wernsman EA, Dougherty WG. Transgenic plant virus resistance mediated by untranslatable sense RNAs: expression, regulation, and fate of nonessential RNAs. *The Plant Cell* 1994;6:1441–53.
- [73] Spielman A, Krastanova S, Douet-Orhant V, Gugerli P. Analysis of transgenic grapevine (*Vitis rupestris*) and *Nicotiana benthamiana* plants expressing an *Arabis mosaic virus* coat protein gene. *Plant Science* 2000;156:235–44.
- [74] Torrance L, Mayo MA. Proposed re-classification of furoviruses. *Archive of Virology* 1997;142:435–9.
- [75] Urban LA, Ramsdell DC, Klomparens KL, Lynch T, Hancock JF. Detection of *Blueberry shoestring virus* in xylem and phloem tissues of high bush blueberry. *Phytopathology* 1989;79:488–93.
- [76] van Dun CMP, Bol JF. Transgenic tobacco plants accumulating *Tobacco rattle virus* coat protein resist infection with *Tobacco rattle virus* and *Pea early browning virus*. *Virology* 1988;167:649–52.
- [77] Vaucheret H, Beclin C, Elmayan T, Feuerbach F, Godon C, Morel J-B, Mourrain P, Palauqui J-C, Vernhettes S. Transgene-induced gene silencing in plants. *Plant Journal* 1998;16:651–9.
- [78] Verchot J, Driskel BA, Zhu Y, Hunger RM, Littlefield LJ. *Soilborne wheat mosaic virus* moves long distance through the xylem. *Protoplasts* 2001;218:57–66.
- [79] Viry M, Serghini MA, Hans F, Ritzenthaler C, Pinck M, Pinck L. Biologically active transcripts from cloned cDNA of genomic grapevine fanleaf nepovirus RNAs. *Journal of General Virology* 1993;74:169–74.
- [80] Visser PB, Mathis A, Linthorst HJM. Tobraviruses. In: Granoff A, Webster RG, editors. *Encyclopedia of Virology*, 3. New York: Academic Press; 1999. p. 1784–9.
- [81] Voinnet O, Pinto YM, Baulcombe DC. Suppression of gene silencing: a strategy used by diverse DNA and RNA viruses of plants. *Proceedings of the National Academy of Sciences USA* 1999;96: 14147–52.
- [82] Wang D, MacFarlane SA, Maule AJ. Viral determinants of *Pea early browning virus* seed transmission in pea. *Virology* 1997;234:112–7.
- [83] Waterhouse PM, Smith NA, Wang M-B. Virus resistance and gene silencing: killing the messenger. *Trends in Plant Science* 1999;4: 452–7.
- [84] Wellink J, van Kammen A. Proteases involved in the processing of viral polyproteins. *Archives of Virology* 1988;98:1–26.
- [85] Wiczorek A, Sanfacon H. Characterization and subcellular localization of Tomato ringspot nepovirus putative movement protein. *Virology* 1993;194:734–42.
- [86] Wisniewski LA, Powell PA, Nelson RS, Beachy RN. Local and systemic spread of *Tobacco mosaic virus* in transgenic tomato. *The Plant Cell* 1990;2:559–67.
- [87] Xiong Z, Lommel SA. *Red clover necrotic mosaic virus* infectious transcripts synthesized in vitro. *Virology* 1991;182:388–92.
- [88] Xiong Z, Kim KH, Giesman-Cookmeyer D, Lommel SA. The roles of the *Red clover necrotic mosaic virus* capsid and cell-to-cell movement proteins in systemic infection. *Virology* 1993;192:27–32.
- [89] Yamamiya A, Shirako Y. Construction of full-length cDNA clones to *Soil-borne wheat mosaic virus* RNA1 and RNA2 from which infectious RNAs are transcribed in vitro: virion formation and systemic infection without expression of the N-terminal and C-terminal extensions to the capsid protein. *Virology* 2000;277:66–75.
- [90] Yepes LM, Fuchs M, Slightom JL, Gonsalves D. Sense and antisense coat protein gene constructs confer high levels of resistance to Tomato ringspot nepovirus in transgenic *Nicotiana species*. *Phytopathology* 1996;86:417–24.
- [91] Zeigler-Graff V, Bouzoubaa S, Jupin I, Guilley H, Jonard G, Richards K. Biologically active transcripts of *Beet necrotic yellow vein virus* RNA-3 and RNA-4. *Journal of General Virology* 1988;69:2347–57.