Status and Prospects of Plant Virus Control Through Interference with Vector Transmission

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Keywords
aphid, hopper, mite, nematode, thrips, whitefly, zoosporic endoparasite

Abstract

Most plant viruses rely on vector organisms for their plant-to-plant spread. Although there are many different natural vectors, few plant virus–vector systems have been well studied. This review describes our current understanding of virus transmission by aphids, thrips, whiteflies, leafhoppers, plant hoppers, tree hoppers, mites, nematodes, and zoosporic endoparasites. Strategies for control of vectors by host resistance, chemicals, and integrated pest management are reviewed. Many gaps in the knowledge of the transmission mechanisms and a lack of available host resistance to vectors are evident. Advances in genome sequencing and molecular technologies will help to address these problems and will allow innovative control methods through interference with vector transmission. Improved knowledge of factors affecting pest and disease spread in different ecosystems for predictive modeling is also needed. Innovative control measures are urgently required because of the increased risks from vector-borne infections that arise from environmental change.
INTRODUCTION

Plant viruses are economically important pathogens and the majority require a vector. A vector is an organism that transmits a virus from an infected to a healthy host by a mechanism that is governed by specific features of virus, vector, and host. Vector: an organism that transmits a virus from an infected to a healthy host by a mechanism that is governed by specific features of virus, vector, and host. Zoosporic: producing zoospores.

VIRAL VECTORS

Host resistance can play a major role in the fight against plant viruses. Several essential steps in the infection cycle can be targeted by resistance genes, but only a few plant resistance genes have been described that operate against vectors or the vector transmission process (18). Characterization of such genes is still incomplete (75). In general, few transmission-associated resistances are known and as a consequence, deployment of host resistance has had limited application in integrated strategies for vector control (115).

The lack of knowledge of these transmission processes is especially serious given that agricultural practices are currently experiencing major challenges posed by environmental change. Global warming leads to greater abundance and increased geographic range of viruses and vectors. Furthermore, the current policies to withdraw or decrease the use of pesticides mean that some important vectors are not efficiently controlled. These challenges pose the risk of increased incidence of vectors and their associated virus diseases.

A study by the European Union (EU)-funded Coordination Action ResistVir concluded that interference in the vector transmission process as a potential target for effective control had to be reconsidered and that such a serious situation needed innovative control measures: These should be both effective and sustainable as well as applicable to a large number of crop plants in different habitats. This review presents a synthesis of recent findings, compares what is known about the characteristics and transmission mechanisms of important classes of vectors, describes recent advances in the field, and highlights gaps in knowledge and areas for future research.

The following sections focus on the features of the best-described plant viral vectors: aphids, thrips, hoppers, whiteflies, mites, nematodes, and zoosporic organisms. For each vector group, we briefly describe their importance in terms of their impact on dissemination of viruses and the current knowledge about the transmission mechanisms. Representative examples are given of viruses transmitted by aboveground (Figure 2a) and belowground vectors (Figure 2b), with further details described below.

CHARACTERISTICS AND IMPORTANCE OF APHIDS AS VIRAL VECTORS

A significant number of plant viruses are transmitted by aphids (98). In nature, aphids are
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<tr>
<th>Mode of transmission</th>
<th>Timeframe</th>
<th>Examples of virus groups</th>
<th>Mechanism</th>
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<td>Acquisition</td>
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<td>min-h+*</td>
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* Depending on number of probes
* CaMV transmission is considered SP or bimodal
* Feeding length correlates with efficiency
* Some rhabdovirids can be transmitted by leafhoppers or planthoppers
* Acquired as larvae/adult
* Larva/adult stages
### Belowground

<table>
<thead>
<tr>
<th>Vector</th>
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<th>Timeframe</th>
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<th>Mechanism</th>
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<td>CP</td>
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<td>Tobravirus</td>
<td>2b + others? Ectoparasitic trichodorid vectors Retention at esophagus</td>
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*Infectivity might be retained during years

**Figure 2**

Schematic representation of some (a) aboveground and (b) belowground vector organisms responsible for the dissemination of selected groups of plant viruses, with indication of the mode of transmission. The time frames of the stages during the transmission process are indicated with sectors on clock faces or with calendar pages. Simplified diagrams of virion morphologies (icosahedral, elongated rigid/flexuous, bacilliform, twinned, etc.) are depicted. Viral products, including the coat protein (CP) and other structural proteins acting during transmission, are indicated. When known, the participation of nonstructural accessory factors is also indicated. The last column includes information on vectors, such as specificity, retention sites, and details about the route of circulation when applicable. For size constraints, several groups of vectors, such as leafhoppers, planthoppers, mealybugs and soft scales, are not represented. Abbreviations: C, circulative; C-Pr, circulative-propagative; NP, nonpersistent; P, persistent; RT, read-through; SP, semipersistent.
present in large numbers thanks to partheno-
genetic reproduction, which can generate
fast, exponential population growth. Their
piercing-sucking mouthparts also help make
aphids efficient vectors. Despite the similarities
of aphids and other hemipterans in mouthpart
anatomy and feeding processes, aphids appear
to be the only vectors that can transmit viruses
after briefly probing a leaf (98), although
aphids also transmit viruses that circulate and
even propagate in aphid bodies.

Most viruses are thought to be transmitted as
intact virions, and in addition to the major coat
protein (CP), many aphid-transmitted viruses
depend on additional, nonvirion proteins for
their transmission. These factors include the
helper component (HC) of potyviruses (108),
the read-through (RT) protein in the capsid of
luteoviruses (150), or the CPm (a minor CP
form) that forms the rattlesnake (RS) portions
of the closterovirus particle (3). Common fea-
tures are that they enable transmission and often
determine vector specificity.

MECHANISMS OF
APHID-MEDIATED VIRUS
TRANSMISSION

Noncirculative Virus Transmission

A majority of aphid-vectored plant viruses are
transmitted in a noncirculative (nonpersistent)
manner that is characterized by short acqui-
sition and inoculation periods in the range of
seconds to minutes and also by short retention
periods. The term semipersistent is used when
the aphid remains viruliferous for from a few
hours up to days. The early literature referred
to nonpersistent viruses as stylet-borne, which
suggested that the virus simply contaminated
the stylet. This idea was abandoned when evi-
dence was presented to demonstrate that a regu-
lated process was essential to retain virions near
the distal tip of the stylets (85). Reversible re-
tention is expected to occur in specific anatomical
structures where the food and salivary canals
merge at the tip of the maxillary stylets: Proteins
located there can act as binding receptors
for caulimoviruses (142). The wide range of
variation in virus transmission efficiencies be-
tween different aphid species might be partially
explained by the specificity of these largely un-
known structures. Salivation has been proposed
to be essential for the release of retained virions.

Two strategies of aphid transmission have been described for noncirculative
viruses: capsid-only exemplified by the cucu-
moviruses and helper-dependent exemplified
by caulimoviruses and potyviruses (110).
Early evidence that the cucumoviral CP alone
contains the determinants for transmission was
obtained when purified virions were acquired
by aphids feeding through artificial membranes
(109) and confirmed using transmissible and
nontransmissible isolates that varied only
in the CP (32). The particle structure of
cucumoviruses has been resolved, and this has
allowed identification of residues and structural
elements essential for transmission (50). Recent
studies found a complex interaction in which
the Cucumber mosaic virus (CMV) suppressor
of silencing might enhance aphid transmission
through alteration of host plant defenses (157).

For caulimoviruses, a semipersistent or bi-
modal mode of transmission has been proposed
(93) with two viral products, P2 and P3, par-
ticipating in the process along with the virions
(76). The interaction between P3 and the viri-
ons creates a complex (112) that may attach to
P2. The subcellular localization of P2 in large
inclusion bodies and its capacity to interact with
microtubules (17) suggest a model in which P2
aggregates to form a transmission body, which
is ready to burst and disperse virions into the
whole cell when the aphid feeds, thus increas-
ing the chances of the virus being acquired (60),
allowing an immediate response to the aphid
presence (87). Interestingly, signals induced by
aphid stylets during penetration into the cell
have been shown to produce alterations in these
microtubule-associated inclusion bodies (86).
Transcomplementation of genomes might ex-
plain why these mechanisms exist: It has been
postulated for Cauliflower mosaic virus (CaMV)
that aphids first acquire P2 from mesophyll
cells, and later they take up P3-virion complexes
from distal parts of the plant (44). Indeed, the requirement of transmission factors has been proposed to have arisen during evolution as an adaptive system to deal with bottlenecks created by the transmission process (108).

For potyviruses, HC is required for retention on the aphid stylettes (152). The structure of HC has only been partially solved (122). Interestingly, the presence of HC in virions, particularly at one end of the particle, might be relevant (140). Mutagenesis allowed identification of essential domains implicated in transmission, including a conserved DAG (aspartic-alanine-glycine) motif located near the N terminus of potyviral CP (11) and one or more specific motifs in the HC related with CP interaction (126). The bridge hypothesis suggests that HC must bind to elements in the aphid vector and proposes a role for specific motifs [e.g., KITC, (lysine-isoleucine-threonine-cysteine)] and conserved residues near the N terminus of HC in these interactions (10, 16). Mutations in the KITC motif alter the capacity of potyviral HC to bind specifically to insect proteins (43).

Other aphid-transmitted viruses encoding determinants for noncirculative transmission can be found among the clustroviruses. In this case, CPm is found in the virus particles and may play a role in the formation of a tail-like structure in the particles (106). However, even for economically important viruses, such as Citrus tristeza virus, information about the transmission mechanism is limited, and only indirect evidence of involvement of structural proteins is available (58).

**Circulative Virus Transmission**

Transmission of circulative viruses is characterized by longer acquisition periods that last from days to weeks. The virus must circulate within the vector through the aphid digestive system to the salivary glands. The latent period is the time between the start of acquisition and the start of the first successful inoculation. The period in which the vector retains the ability to transmit the virus is known as the retention period. Depending on the capacity of the virus to replicate in vector cells, circulative viruses can be classified as propagative or nonpropagative. For those viruses transmitted in a circulative, nonpropagative manner, transmissibility may be maintained for their whole further life, and the virus is not lost through molting. This mode of transmission is found in the Enamovirus, Luteovirus, and Polerovirus genera of the Luteoviridae (53). Being phloem-limited viruses, acquisition of luteoviruses involves ingestion from infected phloem cells. Within the insect, virions travel through the food canal and forget into the mid- and hindgut, where they have to cross cellular barriers, which might impose vector specificity, to access the hemocoel cavity and circulate toward the accessory salivary gland to finally exit in the saliva after passing through a second cellular barrier (51). The existence of these barriers accounts for the specificity of transmission by aphid species, as proven by experimental introduction of virions into the hemolymph (105). The CP of luteoviruses is a determinant of aphid-specific transmission, as shown by trans-encapsulation experiments in double-infected plants (119). The presence of a minor structural protein in virions was later shown to correspond to an RT variant of the CP, which regulates transmissibility and vector specificity (22). The interaction of a CP-RT protein with symbionin, a protein homolog of the GroEL chaperone produced by endosymbiotic bacteria of the genus Buchnera, was considered an essential factor to stabilize virions in the hostile environment of the aphid hemolymph. However, the interaction’s contribution to transmission is controversial because luteoviruses bind symbionins of both vector and nonvector aphids (146), and recent studies on localization in vivo of the chaperone question its availability for interaction (20).

Considering that internalization in the insect cells, with or without replication, is essential for circulative transmission, the existence of specific insect receptors required for the endocytosis of virions, for their transport in vesicles across cells, and for their release by exocytosis has been postulated. Binding assays have been used to identify putative receptors mediating
the transmission of luteoviruses e.g., Barley yellow dwarf virus (BYDV-MAV) in *Sitobion avenae* (80). These constitute putative biomarkers, which might serve to predict the transmission competence of particular aphid species (34).

Luteoviruses can act as helper viruses in the transmission of viruses belonging to the genus *Umbravirus*, which lack CP (133). The association of these viral entities may be even more complex, requiring the presence of satellite molecules that provide assistance for encapsidation and aphid transmission (41).

The circulative propagative virus transmission resembles the dissemination of animal viruses by mosquitoes and other blood-feeding vectors because viruses can replicate in the insect and exit via the salivary glands (59). Plant rhabdoviruses persist in the infected insect for life, and the virus can follow a transovarial route to reach the offspring (116). This family includes viruses that replicate in vertebrates, invertebrates, and plants, and are mainly persistently transmitted by aphids, leafhoppers, or planthoppers (8). Recently, aphid transmission of a reovirus was also demonstrated (114).

**CHARACTERISTICS AND IMPORTANCE OF THRIPS AS VIRAL VECTORS**

Thrips are important vectors of tospoviruses, which include more than 20 species that cause severe disease in both horticultural and ornamental crops (104). *Tomato spotted wilt virus* (TSWV) occurs worldwide, and the host range includes more than 1,350 plant species. *Impatiens necrotic spot virus* infects at least 300 plant species. The viruses are acquired by and replicate in both larval instars and adults, but only the acquisition by the first instar results in transmission by late second instars and adults. The second instar usually reintroduces the virus to the plant on which they emerge or spreads the virus when leaf contact between plants occurs, whereas adults can disperse virus over considerable distances.

Fourteen thrips species are known to transmit tospoviruses (104), but there is wide variation in specificity. For example, *Frankliniella occidentalis* spreads TSWV in tomato and pepper in Europe, whereas *Frankliniella schultzei* and *Frankliniella fusca* are the most common vectors of TSWV in South and North America, respectively. In Southeast Asia, *Thrips palmi* vectors several tospoviruses, including *Watermelon silver mottle virus*, *Melon yellow spot virus*, and *Watermelon bud necrosis virus*, whereas *Ceratobrachyplus claratris* is the vector for *Capsicum chlorosis virus* and *Tomato necrotic spot virus*. *Thrips tabaci* vectors TSWV in tobacco crops in southern Europe but is the only transmitter of *Iris yellow spot virus*. In Europe, the tabaci-type populations of *T. tabaci* are efficient transmitters of TSWV, whereas communis-type populations, which prefer other plant species, were inefficient transmitters (156).

**MECHANISMS OF THRIPS-MEDIATED VIRUS TRANSMISSION**

The various life stages of thrips play different roles in acquisition and transmission of tospoviruses. The life cycle of thrips from egg to adult encompasses two larval stages and two pupal stages. The larvae and the adults ingest food and can consequently acquire viruses. Tospoviruses are propagatively transmitted, so they must replicate in the thrips before they can be transmitted (153). Virus replication occurs in both larval stages and adults.

Surprisingly, only the first instar larvae, and probably a very few second larval instars when they ingest sufficient virus, become transmitters as shown for *F. occidentalis*. Adults and second instar larvae just before pupation also transmit the virus, but their competence to transmit decreases rapidly with the age at which the first instar larvae ingest virus (144).

The median acquisition access and the inoculation access periods vary between one and two hours, whereas maximal transmission rates can be obtained by longer acquisition periods (154). The length of the latent period is temperature dependent and thus related to the rate of the larval development. The transmission
competence differs with the population studied and can vary from 10% to more than 75% for *F. occidentalis* populations that transmit TSWV (145).

Studies on the ontogeny of the internal organs of the thrips have offered a possible explanation for the transport of the virus from the midgut to the salivary glands (94). The developing cibarial muscles in the head of the first instars push the esophageal ganglion into the thorax. As a result, the salivary glands are displaced further into the thorax and make contact with the Mg1 region of the midgut. During this contact, the virus is translocated from the midgut muscle cells into the salivary glands. In the course of further metamorphic development of the wing muscles, the glands are pushed back into the head capsule and as a consequence their contact with the midgut is broken. The rapid decrease of the ability of the first larval stage to become transmitters can be explained by the retraction of the salivary glands before sufficient levels of the virus have been produced in the midgut to seed the salivary glands with the virus. The poor transmission of a deficient isolate consisting of a relatively low number of complete particles can thus be explained by the limited production of virus particles in the midgut (95). Questions remain on how the virus is transported from the midgut to the salivary gland cells.

**CHARACTERISTICS AND IMPORTANCE OF MITES AS VIRAL VECTORS**

Although mites have been linked to several devastating crop diseases, they have only recently been linked to plant virus transmission. Plant virus–transmitting mites belong to the genera *Aceria*, *Cecidophyopsis*, *Brevipalpus*, *Phytoptus*, and *Phyllocoptes*. The *Eriophyoidea* and the *Tetranychoidea* are obligate plant parasites, but thus far no tetranychoids have been found to transmit plant viruses. Mites seem to be the sole vectors for several important viruses, such as *Blackcurrant reversion virus* (BRV) (79), *Citrus leprosis virus* (CLV) (13), *High Plains virus* (HPV), *Pigeon pea sterility mosaic virus* (PPSMV) (91), and *Wheat streak mosaic virus* (WSMV) (127), that cause serious crop losses in cereals, berries, and fruit crops. The mite-transmitted viruses belong to several genera.

The genus *Emaravirus* has recently been established to accommodate a group of viruses with four to eight RNAs enveloped by a double membrane. They show some similarities with the Bunyaviridae. Member species include *European mountain ash ringspot associated virus* (EMARaV), *Fig mosaic virus* (FMV), PPSMV, and *Maize red stripe virus* (MRSV), and two other viruses [*Raspberry leaf blotch virus* (RLBV) and *Rose rosette virus* (RRV)] are putative new members. PPSMV is economically important in Southeast Asia, particularly in the pigeon pea industry in India. High Plains disease, caused by MRSV, induces chlorotic spots or mosaic on wheat and maize, leading to complete yellowing of the plant (62). Remarkably, these viruses are all transmitted by different mite species [EMARaV by *Phytoptus pyri*, FMV by *Aceria ficus*, HPV (MRSV) by *Aceria tosichella*, PPSMV by *Aceria cajani*, RLBV by *Phyllocoptes gracilis*, and RRV by *Phyllocoptes fructiphilus*] (91).

**MECHANISMS OF MITE-MEDIATED VIRUS TRANSMISSION**

WSMV is transmitted in a semipersistent manner by the wheat curl mite, *A. tosichella*. The juvenile stage acquires the virus, and together with the adult stage, which cannot acquire virus, it remains viruliferous for days following acquisition. The efficiency of transmission increases with the length of the acquisition access period and gradually decreases during the inoculation period (127). The virus accumulates in the sac-like posterior midgut, but extraintestinal particles can also be found in the parenchymatous cells around the intestine and in the salivary glands. However, there is no evidence of transovarial transmission. Nevertheless, these findings suggest that the virus may circulate in the vector (103). An HC-protease has been shown to mediate WSMV transmission in ways...
similar to that seen for the aphid-transmitted potyviruses (131). Deletion of as few as 24 nucleotides from the C terminus of the HC eliminated transmission completely (130).

The mode of transmission of BRV by gall mites can be regarded as semipersistent. Microscopic analysis indicated that gall mites can only penetrate the distance of a single epidermal cell (66), and ultrastructural analyses did not reveal nepovirus-like particles inside mites collected from galled buds (118). Moreover, transmission by mites from diseased to healthy test plants can occur in only four hours (141). These data suggest that BRV transmission is not circulative. Other evidence of a semipersistent mode of transmission comes from structural studies and comparisons to other mite-transmitted viruses. Multiple alignments of CP sequences revealed conserved amino acid triplets [STS (serine, threonine, serine) and KAG (lysine, alanine, glycine)] in the BRV RNA2-encoded polyprotein (61). According to the available structural model (28), both STS and KAG motifs occur not only on the surface of CP but also on the surface of virions. KAG is located in the area where five CP domains meet to form a spike outside the virus structure. This triplet is clearly extended from the surface and may serve as a true recognition site for the mites. Interestingly, the triplets STS and KAG are found not only in BRV but also in the CP of Brome streak mosaic virus (BrSMV), BrSMV-H (Hordeum isolate), and Ryegrass mosaic virus (RGMV). KAG has also been found in WSMV CP.

Little information exists on the mode of transmission of CiLV (Citrus leprosis virus) and emaraviruses. The protonymphs, deutonymphs, and adults of Brevipalpus phoenicis acquire and transmit CiLV-C (cytoplasmic type) after circulation through the vector body. The virus is not transovarially transmitted, and there is no conclusive evidence that the virus replicates in the vector (12). The genomic and complementary forms of RNA 3 of EMARaV and the putative P3 nucleocapsid protein were found in its vector P. pyri, suggesting that EMARaV may replicate in P. pyri (91). The short retention period of PPSMV in its vector and the absence of a latent period suggest that this virus is transmitted in a semipersistent manner (72). No transovarial transmission has been observed for PPSMV and MRSV. Given that emaraviruses are transmitted by different mite vectors, they could possibly use different mechanisms of transmission, with some being replicative.

**CHARACTERISTICS AND IMPORTANCE OF WHITEFLIES AS VIRAL VECTORS**

Whiteflies occur in warm climates as pests of both woody and herbaceous plants and in temperate climates mostly as pests of protected crops (see Reference 96). Their importance as pests has been recognized since the end of the nineteenth century (45). Among the 1,300 species of whiteflies, only a few species in the genera *Bemisia* and *Trialeurodes*, and possibly in *Parabemisiae*, are known to transmit plant viruses. However, the global agronomic importance of whiteflies is obvious considering the large number of viruses they transmit.

**MECHANISMS OF WHITEFLY-MEDIATED VIRUS TRANSMISSION**

Whiteflies are phloem feeders, and the known whitefly-borne viruses have semipersistent or persistent relationships with their vectors. Viruses belonging to the *Closteroviridae* or to the genus *Ipomovirus* of the *Potyviridae* are transmitted in a semipersistent manner (97). The precise role of virus-encoded protein(s) and site(s) of virus retention are still under investigation in most cases, but both *Lettuce infectious yellows crinivirus* (LIYV) and *Lettuce chlorosis crinivirus* (LCV) are transmitted from purified virion preparations, indicating that all proteins needed for transmission are structural proteins. Transmission of LIYV is determined by a CPm-mediated virion retention mechanism in the anterior foregut or cibarium of whitefly vectors (30). At least three different proteins seem to be involved in the case of LCV. Begomoviruses are transmitted in a
persistent, circulative manner. The complex interactions of begomoviruses with their whitefly vectors have been recently reviewed (see Reference 59). Begomoviruses circulate in their vector, invading most of the internal organs and exiting from the principal salivary glands (33). To do this, begomoviruses require a functional CP (25). Begomovirus transmission by whiteflies depends on a GroEL homolog produced by coccoid whitely symbionts. It has been suggested that GroEL has a basic and conserved role in the transport of macromolecules in aphids and whiteflies (59). The two approved Torradovirus (Secoviridae) are apparently also transmitted by whiteflies (7).

CHARACTERISTICS AND MECHANISMS OF LEAFHOPPERS, PLANTHOPPERS, AND TREEHOPPERS AS VIRAL VECTORS

Several plant viruses that belong to the Rhabdoviridae, Reoviridae, and Geminiviridae and those that belong to the unassigned genera Marafivirus and Tenuivirus are transmitted by phloem-feeding leafhoppers or planthoppers, whereas only one virus, Tomato pseudoceory top virus, is reported to be transmitted by the treehopper Micratalis malleifera. Most of the vectors prefer grasses as hosts, and as a consequence most of the viruses are transmitted to one or more grass species. Exceptions include Oat blue dwarf virus, a marafivirus that infects flax as well as grasses, and Wound tumor virus, a phytoreovirus that infects only dicots. The rhabdo-, reo-, marafi-, and tenuiviruses are persistently transmitted and propagate during circulation through their vector. Periods of acquisition and transmission can be relatively short, but the efficiency increases with the length of these periods. Between acquisition and transmission a latent or incubation period can be observed. Some of these viruses are transovarially transmitted. The grass-infecting rhabdoviruses are distributed among the genera Cytorhabdovirus and Nucleorhabdovirus, depending on whether they replicate in the cytoplasm or nucleus, and are transmitted by cicadellids or delphacids. Rhabdoviruses infecting dicots are transmitted by aphids, with the exception of Beet leaf curl virus, which is transmitted by the lace bug Piesma quadratum. Viruses in 3 of the 15 genera of the Reoviridae infect plants. The phytoreoviruses are transmitted by cicadellid leafhoppers, and the fijiviruses and oryzaviruses are transmitted by delphacid planthoppers. Recently, Chen et al. (31) have shown the role of tubular structures induced by the Rice dwarf virus to facilitate its spread within its vector, the green rice leafhopper Nephotettix cincticeps. A recently discovered reovirus, Raspberry latent virus, is vectored by the aphid Amphorophora agathonica. Its transmission and genetics support a classification in a new genus designated Raslavirus (114). The tenuiviruses that form an unclassified genus are transmitted by delphacids. All six viruses of this genus infect grass species. Viruliferous delphacids do not only transmit these viruses to plants; the virus is also transmitted to offspring by the females via eggs. The marafiviruses are all transmitted by cicadellids in a propagative manner. The viruses of the genera Mastrevirus and Curtovirus in the Geminiviridae are transmitted by cicadellids. Three viruses of the first genus infect dicots, and the other 12 approved species infect poaceous plant species, whereas the curtoviruses infect only dicots. These viruses are transmitted in a persistent but nonpropagative manner and are not transovarially transmitted. Two members of the genus Waikavirus (Sequiviridae), Maize chlorotic dwarf virus and Rice tungro spherical virus, are transmitted in a semipersistent manner by cicadellids. None of the cicadellids and delphacids has been reported to transmit viruses nonpersistently.

CHARACTERISTICS AND IMPORTANCE OF NEMATODES AS VIRAL VECTORS

Although it has been known since the early part of the twentieth century that plants could acquire virus infections by growing in contaminated soil, the first direct
demonstration that soil nematodes could transmit plant viruses was not reported until 1958. To date, approximately 30 nematode species are known to transmit at least 14 different viruses. These viruses belonged initially to two genera, *Nepovirus* (nematode-transmitted polyhedral viruses) and *Tobravirus* [type species *Tobacco rattle virus* (TRV)] (reviewed in 135). More recent studies have reclassified some of the nematode-transmitted nepoviruses to two new genera, *Cheravirus* and *Sadwavirus*. In a further complication, the majority of nepoviruses have not been experimentally demonstrated to be nematode transmitted, and the sadwaviruses *Blackcurrant reversion virus* and *Strawberry mottle virus* are transmitted by mites and aphids, respectively.

**MECHANISMS OF NEMATODE-MEDIATED VIRUS TRANSMISSION**

Nepoviruses are transmitted by nematodes belonging to the family Longidoridae (genera *Longidorus*, *Paralongidorus*, and *Xiphinema*). Tobraviruses are transmitted by nematodes from the family Trichodorididae (genera *Trichodorus* and *Paratrichodorus*). Both longidorids and trichodorids are ectoparasitic and feed from the outside of the root, most often targeting the region at or near the root tip. Both types of nematodes feed by using a spear-shaped structure located within the esophageal region at the anterior end of the body to puncture the plant cell wall, allowing the cell contents to be extracted, including virus particles if the plant is infected. Using electron microscopy, virus particles have been seen in association with the surface of the spear and surrounding esophageal cavity. It is thought that during feeding some of these particles are released into the root cell, completing the transmission process. The virus is not thought to circulate within the nematode, and particles that are not retained in the esophagus pass into the gut and are excreted. So far, no information is available on the nature of the nematode components involved in binding virus particles or on the composition of nematode secretions that might be required to release virus particles. However, it is suspected that the release process is more important for transmission than the retention process because both Scottish and English strains of *Raspberry ringspot virus* were retained in the nematode *Longidorus macrosoma*; however, only the English strain was transmitted by this nematode.

Nepoviruses and tobraviruses have bipartite genomes and for both, RNA1 encodes replication-associated proteins. Nepovirus RNA2 encodes a replication helper protein, a movement protein, and the CP, whereas tobravirus RNA2 encodes the CP and other nonstructural proteins. Tobravirus RNA2 differs extensively between isolates; however, nematode-transmissible isolates encode the CP, a second protein (2b) that is required for transmission, and sometimes one or more additional proteins that may be involved in transmission by specific vector species.

For tobraviruses, the C-terminal part of the CP is unstructured and required for transmission. The 2b protein is absolutely required for transmission to occur. Yeast two-hybrid experiments have shown that the CP and 2b proteins interact, and by immunogold electron microscopy the TRV isolate PaY4 2b protein was shown to associate with virus particles in leaf extracts. A working hypothesis is that the 2b protein interacts with the nematode esophageal surface and forms a bridge between this and the virus particle. Another tobravirus RNA2-encoded protein, 2c, is required for transmission of *Pea early browning virus* isolate TpA56 but not for transmission of TRV isolate PpK20, suggesting that different combinations of virus proteins are required for transmission by different species of vector nematode (57, 83).

For nepoviruses, the transmission of *Grapevine fanleaf virus* (GFLV) by *Xiphinema index* has been studied in the most detail, revealing that the virus CP is the sole determinant of transmission. Structural modeling of the GFLV CP has identified a positively charged, eleven amino acid domain surrounded by three surface loops that is required for successful transmission. It is hypothesized that...
electrostatic interaction between this CP region and molecular structures on the nematode feeding apparatus may be important for the retention of virus particles and that strategies aimed at disrupting this interaction might be developed for crop protection purposes (125).

**CHARACTERISTICS AND IMPORTANCE OF PLASMODIOPHORIDS AND CHYTRIDS AS VIRAL VECTORS**

A striking feature of soilborne fungal transmission of viruses is that only six vector species have been clearly identified to date. They are all soilborne zoosporic obligate endoparasites belonging either to the protists (three species of plasmodiophorids) or to the chytrid fungi (three species of Olpidium). The plasmodiophorids are vectors of viruses from the families Potyviridae and Virgaviridae as well as from the unassigned genus Benyvirus. *Polymyxa betae* transmits four viruses to sugar beet, *Polymyxa graminis* transmits 14 viruses to cereals and groundnut, *Spongospora subterranea* f.sp. *subterranea* transmits one virus to potato, and *Spongospora subterranea* f.sp. *nasturtii* transmits one virus to watercress. The chytrid fungi *Olpidium brassicae*, *Olpidium bornovanus*, and *Olpidium virulentus* vector fifteen viruses all grouped in the Ophioviridae and Tombusviridae families as well as with the unassigned genus *Varicosavirus* (26). Recently, *Pepino mosaic virus*, a potexvirus, was reported to be soilborne and transmitted by *O. virulentus* (6).

Two modes of transmission have been proposed based on either in vitro or in vivo virus acquisition by zoospores. All soilborne icosahedral viruses except *Watercress yellow spot virus* are acquired externally by *Olpidium*. The viruses with rod-shaped or filamentous particles are acquired from within plant tissues and are carried within the zoospores and resting spores. Soilborne viral diseases have attracted considerable attention because of their major economic impact, which is often linked with their persistence in environmentally resistant resting spores of the vector that can last for decades in soil.

**MECHANISMS OF PLASMODIOPHORID- AND CHYTRID-MEDIATED VIRUS TRANSMISSION**

The study of the in vitro transmission of viruses by *Olpidium* suggests a multistep mechanism with an acquisition and a release step. *Cucumber necrosis virus* (CNV) isolates deficient in zoospore attachment have been identified as well as have nontransmissible CNV mutants that are able to attach to the zoospore. The distribution of virus particles on the zoospore plasmalemma as well as on the surrounding sheath of the flagellum was shown by electron microscopy and indirect immunofluorescence microscopy (102). The binding of virus to zoospores is inhibited by treatment of zoospores with trypsin or periodate (69) or through competition studies, suggesting the presence of some glycoprotein receptors on the zoospore surface. The major role of the virus CP in a specific interaction was demonstrated by the use of chimeric viruses with CP exchanges between transmissible and nontransmissible strains (89, 102). Questions remain about how the virus is delivered into the plant cell. Kakani et al. (68) have shown that virus particles have an altered conformational structure at the surface of zoospores, which may be important for transmission into plant cells.

Concerning in vivo transmission, many reports have shown the presence of virus-like particles within *Polymyxa* (82), but there are contradictory reports claiming the detection of movement protein and nucleic acids of *Soilborne wheat mosaic virus* (SBWMV) within the sporosores (71). *Beet necrotic yellow vein virus* (BNYVV) particles have been observed inside zoospores. Spontaneous deletion mutations that impair vector transmission occur in the CP-RT domains of BNYVV, SBWMV, *Soilborne cereal mosaic virus* (SBCMV), *Oat golden stripe virus* (OGSV), and *Potato mop-top virus* (PMTV) as well as in P39 of *Peanut clump virus* (2, 134). Furthermore, the CP-RT domains of BNYVV, *Beet soilborne virus*, *Beet virus Q* (37), PMTV, SBWMV, SBCMV,
and OGSV, as well as the P2 of bymoviruses, possess at least two transmembrane domains sharing conserved helices. This has led to the hypothesis that these structural features may be involved in vector transmission (42). Interestingly, a KTER (lysine-threonine-glutamic acid-arginine) motif positioned within one of these two domains was mapped as a key feature of the interaction of BNYVV with *P. betae* (134). However, one cannot rule out a process involving multiple viral proteins: For example, the P31 encoded by BNYVV RNA-4 and the P32 of BSBMV as well as the CP-RT are involved in the *P. betae* transmission (38).

### AVAILABLE CONTROL MEASURES FOR VECTOR-MEDIATED VIRUS TRANSMISSION

Control of virus vectors is often dependent on the use of pesticides. Deployment of host resistance through exploiting resistance genes or by genetic engineering is also an important strategy in vector control. These methods have advantages and disadvantages for incorporation in integrated management strategies that are described in the following sections.

#### Chemical Control

The intensive use of pesticides against vectors to control plant virus epidemics is a strategy that has many adverse environmental effects and consequently is being increasingly questioned (27). In certain cases, the effect of treatments can even enhance the dispersal of the viruses, as described for aphids transmitting potyviruses that infect potato (136). Similarly, the use of insecticides to control insect vectors is often considered to be ineffective in suppressing tospovirus infections (104), especially in annual crops. The small time interval during which insects can acquire and subsequently transmit viruses generally explains the poor efficiency of insecticides in controlling nonpersistently transmitted viruses.

New legislation is challenging vector control by banning chemicals that have been essential in the elimination of vectors. For example, soil treatment with zinc compounds is known to suppress zoospores of the causal agent of potato powdery scab (*Spongospora subterranea*), and resting spores of plasmodiophorids can be eliminated by application of carbofuran (40) or fumigation with methyl bromide (56) or dichloropropene/propane (21). Although increases in sugar beet yields following such treatment to control rhizomania disease were obtained, the use of these products is being phased out because of health hazards and adverse effects on the environment associated with their use. In Europe, organochlorine compounds as well as nematicides and soil fumigants have been withdrawn following EU legislation, and chemical measures to control plant pests are restricted to a limited number of substances (e.g., sulfur sprays and endosulfan).

Alternatively, environmentally friendly methods that are safe for consumers have been proposed against zoosporic vectors. These methods include the use of compost-like azidarachtin (4) or salicylic acid (23) and the use of microorganisms, such as *Pseudomonas fluorescens* (117), *Pseudomonas putida* (5), *Streptomyces* sp. (151), and *Trichoderma* sp. (100), either in soil mixtures or as seed dressings, but their efficiency in reducing BNYVV soil inoculum for rhizomania control on a large scale is much discussed (88). Moreover, questions remain about the effectiveness of such treatments when vector inoculum levels in the soils are depressed rather than eliminated. For example, the disease severity of powdery scab is not correlated with levels of soil inoculum (143). Biological control is normally considered as a good alternative, especially for protected crops, but although the use of natural enemies, such as predators and parasitoids, may reduce populations of potential vectors, they often do not prevent the transmission of viruses.

#### Integrated Management

Integrative and sustainable strategies are being evaluated and implemented that can optimize
chemical usage and combine it with host resistance and other control methods. Integrated management includes certification schemes and use of virus-tested seed to avoid the release of infected plant material contributing to primary infection foci (120). As many crop species can be infected by several viral pathogens, multiplex real-time polymerase chain reaction assays or micro/macroarray detection methods must be developed; for example, a method to detect up to 52 virus species has been devised (99), and macroarrays enable multiplex detection of 35 grapevine viruses (137). Along with sensitive diagnostic methods in certification schemes and improved sanitation methods (70), complementary measures, including, for instance, roguing of symptomatic plants, should also be included as these have had demonstrated utility in specific cases (138). Cultural and physical approaches to combat vectors can include mulching crops (48), using nets and UV-reflecting plastic against flying insects (9), growing crop varieties with durable resistance to viruses or vectors that rely on different mechanisms, and pyramiding host-resistance genes (65, 139). The impact of factors involved in the spread of viral diseases in a given landscape should be assessed to develop appropriate risk assessment and integrated control strategies (e.g., the combination of different resistant varieties, planting dates, plant densities, row pattern, control of vectors in weeds surrounding the fields, application of treatments, and knowledge of disease history) (29). Such integration of different control methods relies also on socioeconomic issues, such as the coordination of actors in a given territory. In the future, more efforts should be directed to collecting data that can be used to inform predictive mathematical models to enable control measures to be applied in a sustainable way (67).

Breeding for Vector Resistance

Resistance to vectors and viruses remains unquestionably the best strategy for disease control, keeping in mind that plant resistance genes represent highly precious resources and that a sustainable management of these resistance genes and quantitative trait loci for pests and diseases is required. Advances in breeding and molecular marker technology have allowed the use of some vector resistance genes. Resistance to wheat curl mite, the vector of WSMV, has been obtained by introgression of a resistance gene from *Aegilops tauschii* into wheat (84). At least five different genes for resistance to mite colonization may occur in *A. tauschii* accessions, suggesting this species is useful for wheat improvement.

The *Mi-1.2* gene identified in tomato plants confers resistance to whiteflies (particularly to *Bemisia tabaci*) (101), to root-knot nematode (121), and to some potato aphids (92). The *Mi-1.2* encodes a cytoplasmic protein with a putative coiled-coil nucleotide-binding site–leucine-rich repeat domain (92). Other genes have been identified as necessary for *Mi-1.2* functionality: *Rme1* (39), *Sgt1*, and *Hsp90-1* (15). The complex of these genes is effective against *B. tabaci* B- and Q-biotypes, although they are more effective against the latter (101).

Cotton (*Gossypium* spp.), tomato (*Solanum lycopersicum*), cassava (*Manihot esculenta*), cucurbits, and a number of *Fabaceae* have been investigated for resistance to whitefly infestation. Leaf trichomes have been investigated as a possible source of resistance to whiteflies (24, 74). Van Lenteren et al. (147) proposed that less hairy (cucumber) plants allow finding and killing of more whiteflies per unit of time by parasitoids, thus improving natural biological control and reducing whitefly population density. The presence of glandular trichomes is an important factor for whitefly resistance. In general, there is a negative correlation between the density of glandular trichomes and the level of whitefly infestation (123). Trichome exudate is responsible for nymph and adult antibiosis (49).

Studying the feeding behavior of vectors might lead to a better understanding of the transmission processes (47). For example, classical tests for antixenosis (found in host preference tests) and antibiosis under no-choice
conditions (128) as well as electrical penetration graphs (EPGs) have been used to compile ranked lists of acceptance of vectors (77) or even to relate the feeding behavior of whiteflies to the presence of the Mi-1 gene (63). Also, phloem sieve elements have been identified as being important for resistance to *B. tabaci* in alfalfa (*Medicago sativa*) (64). For longidorid ectoparasitic nematodes, some wild species of grapevine (*Vitis rotundifolia*) exhibit high levels of dominant resistance toward *X. index* and have been included in breeding programs. A *X. index* partially resistant rootstock (Nemadex AB) that shows a good efficacy in fanleaf-pathosystem-infested soils is being used in French vineyards (46). Control of *X. index* populations by the use of fallow plants, considered as nematoidal plant species, has been demonstrated under greenhouse and vineyard conditions. Plant species belonging to the *Fabaceae* family can reduce the fanleaf inoculum in an infested soil, leading to a significant reduction of the fallow period between successive grapevine plantings (149).

With regards to fungal-associated transmission, resistance to the vector was not considered of major importance because of the ubiquitous presence of *Polymyxa* in soils. However, host resistance to zoospore-borne viruses is either partial or monogenic, together with several reports of resistance-breaking isolates in the United States (1) and Europe (107), giving new motivation to the search for sources of vector resistance.

**CONCLUDING REMARKS AND PROSPECTS FOR FUTURE RESEARCH**

In the future, vector or virus resistance may result in inbred or genetically engineered lines expressing natural resistance genes. Transgenic approaches will likely provide opportunities for introducing new traits to control viruses and/or vectors when no resistance genes are available in related species or to reinforce partial or quantitative resistance, as exemplified by the use of specific sequences targeting vectors or viruses operating via RNA silencing (132). A new mechanism of resistance that relies on a serine hydroxymethyltransferase-encoding gene against the soybean cyst nematode has been recently discovered (81), opening new perspectives for resistance toward nematode vectors. Transgenic approaches are only feasible if transformation and regeneration of transformed tissues is successful, which is currently difficult with woody plants (e.g., *Ribes* and grapevine). Many promising results have shown that engineered resistance against plant viruses through RNA interference (36, 113) could help to achieve sustainable and integrated control of viruses. Other strategies relying on antibodies expressed in transgenic plants, such as plantibodies, have been explored (52). Nanobodies, a new type of antibodies that lack the light chain (as well as antiviral variable antigen-binding domains, which have been shown to inactivate human DNA or RNA viruses) may have the potential to target plant viruses (148).

The application of RNAi technology to different organisms, including insects, is another promising field of research (14). Control of vectors is now feasible with demonstrated examples in other viral pathosystems (e.g., mosquitos and human/animal viruses). For example, using densovirus-mediated RNA interference for aphid control could be a promising strategy, as it is already used to control *Aedes albopictus*, which vectors the Dengue disease (54). Knockdown of particular aphid genes would be feasible after feeding the aphids on plants expressing complementary double-stranded RNAs (111) or on plants infected with virus-derived vectors, inducing RNAi to target genes that are essential for survival of the insect (73).

Recent advances in technology, such as the availability of full-length infectious virus clones for reverse genetic approaches and 3D reconstruction of viral particles at Å resolution to enable structural insights into viral determinants (124), will enable us to investigate the molecular mechanisms of transmission in fine detail. In addition, new technologies (omics) will provide new opportunities to unravel
the mechanisms of virus transmission. For example, the availability of complete genomes together with the ongoing sequencing efforts on important vectors of plant viruses (such as aphids, whiteflies, and nematodes) is providing resources and postgenomic tools that are highly desirable for functional analysis. A major breakthrough in knowledge will happen when receptor-like determinants within the vector to which virions could bind are identified. A few attempts to identify such receptors in aphids, planthoppers, and thrips have been described to date (90, 155). This knowledge will be of major value in elucidating the plant virus–vector interactions.

Studying the host-virus interactome is also critical to shedding light on protein interactions required in the different steps of the transmission process. It could provide insights in the biochemical mechanisms required for the movement in plants and aphids as was shown for luteoviruses. Cilia et al. (35) discovered that sodium sulfite–treated virions were not acquired by aphids through the hindgut epithelial cells and were not transmitted when injected directly into the hemocoel, thus reducing transmission rates by aphids. They showed that particular plant proteins accumulated to higher levels in aphids that fed on virus-infected plants. Their findings suggested a potential role for virus proteins in modulating host plant phloem protein expression to favor the virus uptake by the aphid. These facilitating-vectoring proteins should be examined in-depth as new targets for virus resistance by disrupting phloem-virus-vector interactions (35).

Vector behavior studies are essential to further our understanding of virus-vector interactions; for example, recent data elucidated an unexpected alteration of vector feeding behavior to be to the advantage of the virus they transmit (129, 157) and modifications of various vector life parameters were instigated by the presence of virus (19). Combined with the identification of virus receptors in vectors, this research will shed light on the molecular mechanisms of transmission and enable new discoveries that will help in the understanding of these intimate molecular interactions. This deeper understanding can be used to devise innovative control strategies to disrupt this complex association between host plant, vector, and virus.

Finally, public debate and social concerns (including, for example, the EU legislation on the use of GMOs) might result in limitations to implement further progresses on transgenic approaches for resistance breeding. Interactive technology assessment (78) and environmental risk assessment are needed to integrate genetic engineering in a broad-spectrum, durable, and sustainable control of virus diseases and epidemics spread by vectors.

**SUMMARY POINTS**

1. Most plant viruses rely on vectors for their transmission, and this is an essential part of virus dispersal mechanisms.
2. Large gaps exist in the available knowledge on molecular mechanisms of transmission: Vectors are highly diverse. This review demonstrates that the present knowledge of virus vector-virus-host complexes is limited to a small number of well-studied systems.
3. New methodologies are providing new approaches to discover vector receptors, host vector resistance genes, and many more molecular details of host-virus-vector interactions.
4. Current control strategies that focus on interference with virus vectors include chemical control methods, integrated management approaches, and the breeding of crops with resistance to vectors or low vector preference for the host.
FUTURE ISSUES

1. Basic information on the virus-vector interaction process is lacking in many cases. New approaches based on high-throughput, mass data analysis technologies will contribute to a better understanding on how viruses exploit their vectors for efficient dissemination and on how vectors benefit from the virus they transmit.

2. A better knowledge of virus-vector population ecology is also needed to understand how changes in ecosystems induced by both climate and crop practice modifications may affect disease incidence and transmission.

3. More efforts are needed to discover, analyze, and integrate vector resistance in plants by conventional and/or transgenic approaches, including the use of RNAi to target vectors directly.

4. Integrated control strategies using different approaches, such as deployment of resistance, improved knowledge of the epidemiology of vector populations, disease pressure forecasting, and predictive modeling, are required.

5. Improved disease control strategies must include a consideration of land and crop management, including control of vectors in neighboring vegetation prior to planting and monitoring of vector populations through improved knowledge of vector life cycles in specific agroecosystem.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

DEDICATION AND ACKNOWLEDGMENTS

The authors dedicate this paper to the memory of our colleague Rob Goldbach, who could not contribute to the final version, although he was among the instigators of the original paper. The participants in the different expert groups gathered from the EU-funded project ResistVir are acknowledged for their assistance.

LITERATURE CITED


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125. Described the derivation of the crystal structure of nematode-transmitted nepoviruses (GFLV and ArMV) and cryoelectron microscopy modeling to predict a positively charged cavity that could be important for interaction of the virus with the vector nematode. Mutagenesis of regions of the CP highlighted by the structural analysis identified residues that are essential for successful transmission.


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