Plant Virus Transmission by Insects

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Most plant viruses depend on insect vectors for their survival, transmission and spread. They transmit plant viruses by two principal modes, circulative (circulating through the insect's haemocoel, CV) and non-circulative (carried on the cuticle lining of mouthparts or foregut, NC). Transmissibility and specificity between NC viruses and their vectors depends on the coat protein (CP) of the virus in addition to virus-encoded helper proteins. Circulative viruses cross the gut, circulate in the haemocoel and cross the salivary glands to render the insect infective. Circulative luteoviruses depend on small CP and the read-through protein (RTD) for transmission. Electrical penetration graphs have provided evidence on insect feeding behaviour and virus transmission. Recently, studies have shown that viruses can modify vector behaviour in a way that transmission is enhanced. Cultural, physical and novel biotechnological tools can provide virus control by interfering with vector landing and the retention of viruses in their vectors.

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Introduction

Insect vectors of plant viruses are found in 7 of the 32 orders of the class Insecta. Hemipterans are by far the most important virus vectors, comprising more than 70% of all known insect-borne viruses. Among these, aphids and whiteflies are the major vectors of plant viruses transmitting more than 500 virus species. Two major classifications of viruses have been proposed: attending to the time the vector remains viruliferous [persistent, semi-persistent (SP) or non-persistent (NP)] or the route of the virus within its vector [non-circulative (NC) or circulative (CV)]. More recently, a third classification was proposed based on the localization of virus–vector retention sites: cuticula-borne or salivary gland-borne. A number of viral and insect proteins have been found to control some virus–vector association, but many remain unknown. Interference with vector landing by manipulation of insect vision together with novel molecules that outcompete viruses from the retention sites in their vectors could help reducing plant virus epidemics.

The Importance of Insect Vectors

Most plant viruses depend on vectors for their survival for two principal reasons:

1. An impermeable cuticle coats the plant epidermis, preventing entry of virus particles (animal viruses enter readily through natural openings). Most vectors are insects (non-insect vectors include mites, nematodes and fungi). Several plant viruses may spread by contact or vegetative reproduction. Many insects such as hemipterans are well adapted to their role as vectors by their capacity to pierce the epidermis and delicately deposit the virus in the cytoplasm without risking the integrity of the plant cell. Recent findings propose that viruses have adapted to their vectors modifying their behaviour to maximise their own spread.
2. Plants are rooted and lack independent mobility. Therefore, many viruses depend on insects for transport among hosts (unlike animals that, by their own mobility, transport the virus to new niches).

Insect-borne plant viruses may cause severe or even crippling losses to many annual and perennial crops. On occasion, insects are responsible for transition from a non-spreading form to the epidemic form of diseases. Outbreaks of disease caused by insect vectors are demonstrated in two examples. In perennials, the almost total destruction of the citrus industry in the 1930s in Argentina and Brazil is attributed to the aphid *Toxoptera citricida*. In annuals, outbreaks of *Tomato spotted wilt virus* (TSWV) or begomoviruses in recent decades is attributed to the spread of the thrips *Frankliniella occidentalis* and the whitefly cryptic species complex, *Bemisia tabaci*, respectively.

Taxonomy

Insect vectors of plant viruses are found in 7 of the 32 orders of the class Insecta. The majority of vectors are found in the two orders of insects with pierce-sucking mouthparts (number of species in parenthesis): Hemiptera (300) and Thysanoptera (6). Other vector species are found in five orders of chewing insects: Coleoptera (30), Orthoptera (10), Lepidoptera (4), Diptera (2) and Dermaptera (1).

Mechanisms of transmission

Progress in the molecular biology of viruses and their vectors has assisted greatly in the localization of virus retention sites in their vectors and in identifying motifs in the viral genome and in viral and vector proteins, thus adding to the understanding of the process of virus transmission by insects.

The Major Transmission Modes: Persistent Versus Non-persistent; Circulative Versus Non-circulative

Plant viruses demonstrate a high level of specificity for the group of insects that may transmit them (a virus that is transmitted by one type of vector will not be transmitted by another). CV viruses that propagate in their insect vectors are not considered in this article. The list of the major insect-borne virus groups and their vectors is summarised in **Table 1**.

Modes of transmission

In the 1930s, Watson and Roberts proposed modes of virus transmission by insects. The basis for their assigning viruses to these modes was the duration of virus retention in the vector. Originally, they proposed two modes: NP for short retention or 'less than the time the virus survives in leaf extracts'; and persistent for extended retention, often for life. However, several viruses showed an intermediate retention in their vector. This led Sylvester to designate the term SP viruses (cited in the study by Raccah, 1986). In time, a different terminology was proposed for modes of transmission, based on the site at which the virus is retained in the insect. Thus, NP viruses were termed stylet-borne, whereas persistent viruses were termed CV. In time, additional attributes were attached to each of the modes of transmission (**Table 2**). NP viruses are acquired and inoculated during brief probing times, do not require a latent period in the vector and are transmitted by many aphid species, mostly by those not colonising the crop. SP viruses need longer periods (hours) for acquisition and transmission than do NP viruses. They have a narrower range of vector species. However, they do not require latent period and are lost when the vector moults. In persistent viruses, several hours or even days are needed for efficient acquisition and inoculation. They have a narrow range of vectors, mostly those that colonise the crop, pass through moult and need a latent period.

Many thorough biological, microscopical, immunological, molecular techniques and electronic monitoring feeding devices have subsequently been used to elucidate the mechanisms of transmission. Two principal modes of transmission emerged: (1) CV or internal, where the virus crosses gut barriers and enters the circulatory system of the insect and accumulates inside the salivary glands, and (2) NC or external, where the virus remains attached to the cuticle of the insect mouthparts or foregut and does not cross gut barriers.

The Mechanism of Non-persistent Transmission

Virus particles, but not their naked nucleic acids, are the pathogenic units that are transmitted by insects to initiate infection (Ng and Falk, 2006). On the other hand, viral nucleic acids (either DNA or RNA) are sufficient to cause infection when introduced to plant cells by artificial means (rubbing, bombardment,

Table 1 Major groups of viruses and insect species that serve as vectors

Virus groups	Mode	Persistence	Localization	Insects involved
Alfamovirus	NP	Few hours	Stylets	Aphids
Badnavirus	SP	Days	Unknown	Mealybugs and leafhoppers
Begomovirus	P	Weeks	Salivary glands	Whiteflies
Crinivirus	SP	Days	Foregut/Cibarium	Whiteflies
Carlavirus	NP	Few Hours	Stylets	Aphids or whiteflies
Caulimovirus	NP	Many hours	Acrostyle	Aphids
Closterovirus	SP	Many hours	Foregut	Aphids or mealybugs
Comovirus	SP	Days	Unknown	Beetles
Cucumovirus	NP	Few hours	Stylets	Aphids
Curtovirus	P	Weeks	Unknown	Leafhoppers
Enamovirus	P	Weeks	Salivary glands	Aphids
Fabavirus	NP	Few hours	Stylets	Aphids
Ipomovirus	SP	Days	Unknown	Whiteflies
Ilarvirus	P	Days	Unknown	Thrips
Luteovirus	P	Weeks	Salivary glands	Aphids
Machlomovirus	SP	Many days	Unknown	Leafhoppers
Macluravirus	NP	Few hours	Unknown	Aphids
Mastrevirus	P	Weeks	Unknown	Leafhoppers
Nanovirus	P	Weeks	Salivary glands	Aphids
Potyvirus	NP	Few hours	Stylets	Aphids
Sequivirus	SP	Few hours	Foregut	Aphids
Sobemovirus	SP	Days	Unknown	Beetles
Torradovirus	SP	Days	Stylets	Whiteflies
Tymovirus	SP	Days	Unknown	Beetles
Waikavirus	SP	Few days	Foregut	Leafhoppers

NP, non-persistent; SP, semi-persistent; and P, persistent.

Table 2 Principal characteristics of the modes of virus transmission by insects

Feature	*External (non-circulative)*		*Internal-circulative*
	Non-persistent	Semi-persistent	Persistent[a]
Duration of retention	Brief (few hours)	Intermedia (few days)	Long (days to months)
Duration of acquisition and transmission	Brief (seconds)	Intermediate (hours)	Long (hours to days)
Latent period	Not required	Not required	Required
Tissue where virus is acquired and inoculated	Epidermis and parenchyma	Epidermis, parenchyma and phloem	Mostly phloem
Pre-acquisition fasting	Increase transmission	No effects	No effect
Passage through moult	Negative	Negative	Positive
Insect species specificity	Low	Intermediate	High
Sequential inoculation	Poor	Intermediate	Good

[a] All circulative viruses except *Pea enation mosaic virus* (PEMV) are transmitted in a persistent manner. PEMV is assisted by an umbravirus that allows the virus to invade tissues other than phloem and duration of the acquisition and inoculation periods is similar to viruses transmitted in a non-persistent manner.

agro-infection, etc.). This suggests that protein molecules encapsidating the nucleic acid are needed to interact with specific sites present in the vector. Investigation of the role of the coat protein (CP) in virus transmissibility was possible due to the occurrence of virus strains that differ in their specificity for vector species and the occurrence of strains that have lost transmissibility after continuous mechanical inoculation (see details in the following sections). Recently, the precise location and chemical nature of the first NC virus receptor within the vector mouthparts has been identified. In addition, the specific probing behaviour activities of insect vectors linked to the transmission of plant viruses have also been elucidated with the help of electronic devices.

The Role of the Capsid Protein in the Transmission of Non-persistent Viruses

Cucumoviruses

For *Cucumber mosaic virus* (CMV), Gera and co-workers provided evidence that the genome of a poorly transmissible strain became transmissible when encapsidated *in vitro* with the CP of highly transmissible strain (Perry, 2001; Raccah *et al.*, 2001; Pirone and Perry, 2002). A follow-up of these studies was achieved by Perry and co-workers who designed chimaeric RNA 3 cDNA constructs in order to introduce mutations in the CP (cited in the study by Perry, 2001). As a result of these studies, three amino acid mutations in the CP were found to affect transmission of CMV by *Aphis gossypii*. In a more recent study, these authors discovered that the transmissibility of CMV by *Myzus persicae* requires two mutations in the CP (in positions 25 and 214) in addition to the mutations in positions 129, 162 and 168 that were reported in their former study (Perry, 2001). Charge alterations in the metal-ion-binding βH–βI loop exposed at the surface some non-transmissible CMV mutants are thought to be responsible for the disrupted virus–vector interaction (Liu and co-workers, cited in the study by Ng and Falk, 2006).

Potyviruses

In order to identify the determinants of potyvirus transmission by aphids, the amino acid sequences of the CP of aphid-transmissible (AT) and non-aphid-transmissible (NAT) virus strains were compared. The comparison revealed a conserved amino acid triplet, Asp-Ala-Gly (DAG) within the highly non-conserved and exposed amino terminal end of the CP. The NAT strains were found to have a mutated triplet. A mutation from Gly to Glu (DAG to DAE) was introduced in the CP of an AT strain of *Tobacco vein mottling virus* (TVMV), rendering it non-transmissible. The role of the DAG motif of the CP in aphid transmission was then confirmed also for an NAT strain of *Zucchini yellow mosaic virus* (ZYMV) by changing Thr to Ala (DTG to DAG), this time restoring transmissibility. Effects on transmission of TVMV were noted not only for the DAG triplet but also for amino acids in its immediate vicinity (several studies all cited in the study by Raccah *et al.*, 2001).

Electron microscopic studies provided evidence that the DAG motif in potyviruses is involved in retaining the virus in the aphid's mouthparts. The mechanism is apparently via an interaction of the DAG with a virus-encoded protein named helper component (HC), as shown by the protein-blotting overlay technique (reviewed by Raccah *et al.*, 2001).

Potexviruses

Potato aucuba mosaic virus (PAMV) is not transmissible by aphids, but its transmission is possible when it is assisted by potyviruses. The DAG motif of the CP sequence of PAMV is not present in *Potato virus X* (PVX) but transfer of the DAG motif from PAMV to PVX, resulted in its becoming aphid transmissible (reviewed by Raccah *et al.*, 2001).

Virus-Encoded Proteins That Affect Non-circulative Virus Transmission by Insects

The vectors of potyviruses and caulimoviruses cannot transmit purified virus particles unless these are presented in mixture with a non-structural virus-encoded protein (Blanc *et al.*, 2001; Raccah *et al.*, 2001).

Potyviruses

The helper phenomenon was first reported by Kassanis and Govier (cited in the study by Raccah *et al.*, 2001) showing transmission of the NAT virus PAMV became transmissible in the presence of transmissible *Potato virus Y* (PVY). Later, they demonstrated that potyvirus transmission requires an HC in addition to the virus particles. In addition, they showed that transmission occurs only if the virus is acquired in mixture or after the acquisition of the HC. This led to the formulation of the 'bridge' hypothesis, where the HC binds to aphid mouthparts on one side and to virions on the other; thus, ensuring virus retention until release into the next host. Sequencing of the potyviral genome and identification of the resulting protein assisted in characterising it as a non-structural protein encoded by the HC-Pro region of the potyvirus genome. The helper function in transmission was assigned to the *N*-terminal and central regions of the HC-Pro. The HC proteins have a predicted molecular mass ranging between 50 and 60 kDa. The proposed biologically active form is a dimer. The domains that are involved in vector transmission were traced by comparing strains with active and inactive HC. For TVMV, the loss of HC activity was associated with a mutation in the highly conserved Lys-Ile-Thr-Cys (KITC) motif where Lys was changed to Glu (E to K). This mutation was also present in other potyvirues (mutants of PVY and ZYMV HCs). The KITC motif of the HC is not involved in binding to virions [transmission-defective ZYMV-Ct with K instead of E in the KLSC motif was bound efficiently to virions in overlay blotting experiments (reviewed by Raccah *et al.*, 2001)].

Another conserved motif in the central region of the HC-Pro gene, Pro-Thr-Lys (PTK), was found to be associated with HC assistance in transmission of ZYMV. A mutation from Pro to Ala in the PTK motif resulted in loss of helper activity. The PTK motif was found to affect the HC binding to virions in overlay blotting experiments (reviewed by Raccah *et al.*, 2001). A proposed model summarising the interaction among the virions, the HC and the aphid stylets is depicted in **Figure 1**.

The role of the HC in retaining the virus in the stylet was shown by comparing aphids fed on mixtures of transmissible TEV or TVMV virions and functional PVY HC or TVMV HC (motif KITC) with those fed on non-functional HC (motif EITC) (reviewed by Raccah *et al.*, 2001).

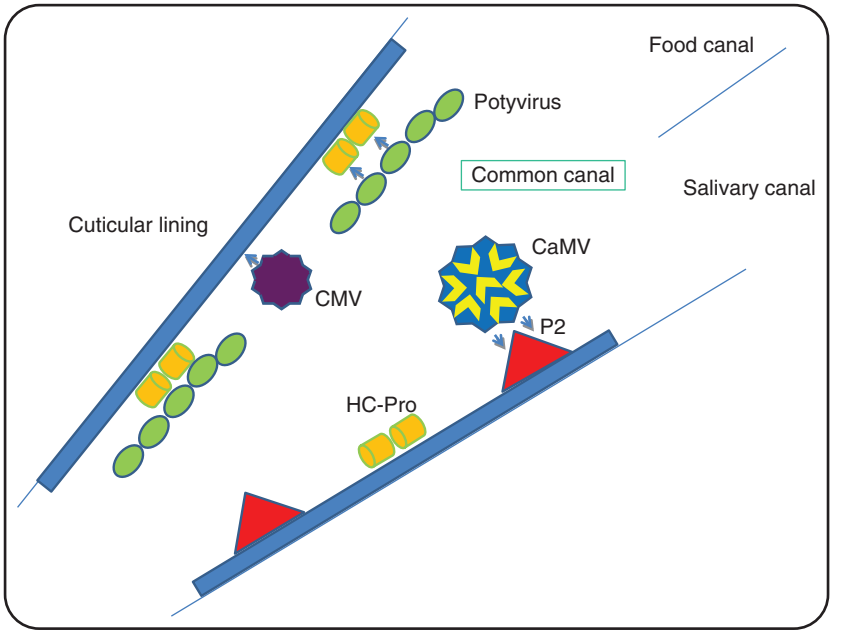

Figure 1 Model describing the different strategies for virus–vector interaction in non-circulative transmission by aphids. These strategies enable retention of virus particles on the common canal of the maxillary stylets at the surface of the cuticular lining. In the capsid strategy, CMV, a motif of the coat protein directly binds to the vector's receptor. In the helper strategy used by potyviruses, virus–vector binding is mediated by the helper component (HC-Pro), which creates a 'molecular bridge' between the two. HC-Pro can be acquired alone or together with the virion. Caulimoviruses (CaMV) also use the helper strategy, but a different protein (P2) acts as a bridge between the virus and the vector.

Caulimoviruses

Caulimoviruses have also adopted a helper-dependent transmission strategy, but in a rather more complex manner than potyviruses. *Cauliflower mosaic* virus (CaMV) requires two viral-encoded non-structural proteins, P2 and P3. A P2-P3-virion complex is formed, with P2 binding to the aphid, whereas P3 binding to the virions (Drucker *et al.*, 2002). Furthermore, the HC motif directly involved in specific vector recognition was identified at position 6 of the N-terminus of P2. A single mutation of one amino acid that may appear spontaneously changes the spectrum of vectors transmitting CaMV (Moreno *et al.*, 2005). Recently, Martinière *et al.* (2013) found that the formation of transmission-specific inclusion bodies of CaMV are not acquired by their aphid vectors, but rather, they react immediately to intracellular stylet punctures and transiently dissociate, forming transmissible P2-virion morphs throughout the cell that increase the acquisition success of the virus.

Indirect evidence suggests that helper is involved in several other systems. The semi-persistently transmitted *Parsnip yellow fleck virus* is not transmissible by aphids unless acquired with the *Anthriscus yellows virus*. A dense material with virus-like particles was seen in aphids' mouthparts after acquisition of the virus. *Rice tungro spherical virus* (RTSV) is transmissible by several *Nephottetix* leafhopper species. RTSV assists the transmission of a second virus, the *Rice tungro bacilliform virus*. In addition, *Maize chlorotic dwarf virus* is semi-persistently transmitted by leafhoppers and is considered to have HCs (cited in the study by Hull, 1994). Lack of vector transmissibility of purified virions led to the speculation that a helper is needed for transmission of carlaviruses and closteroviruses (Raccah *et al.*, 1990).

Mode of transmission of viruses by beetles

Beetle vectors of plant viruses are known in four families (Chrysomellidae, Coccinellidae, Curculionidae and Meloidae) (Gergerich, 2001). Beetle-borne viruses have a unique mode of transmission. The viruses are transmitted in the beetle's regurgitant and there is no latent period in the vector. The original assumption was that regurgitant components selectively inactivate particles of beetle non-transmissible viruses. However, mixing preparations of a variety of viruses with beetle regurgitant had insignificant effect on most viruses (beetle-borne or not). Some beetle-borne viruses are CV, as they were found to move into the insect haemolymph immediately after ingestion. Beetles can also be rendered viruliferous by injecting virus into the haemolymph. However, Wang and co-workers found that beetles may transmit viruses even if they are not carried in the haemolymph. The retention of inoculativity of beetles differs for different beetle vectors; thus, *Epilachna varivestis* retains *Cowpea severe mosaic virus* for 1 day, whereas *Cerotoma trifurcata* transmitted the same virus for several days. The virus does not propagate in the beetle as the virus titre declines with time. Gergerich and co-workers demonstrated the unique role of the regurgitant in the infection process. Viruses not transmissible by beetles were mechanically infectious to wounded hosts, but when

regurgitant was added to the inoculum mixture, only beetle-borne viruses remained infectious. The inability of virus particles to infect hosts was not due to inactivation since, when purified away from the regurgitant virus particles regained infectivity. This finding suggests that an inhibitor in the regurgitant affected the host itself or the interaction between virus and host and that viruses transmissible by beetles differ from other viruses in the fast translocation to non-wounded cells through the xylem and in the manner in which they initiate primary infection.

The Mechanism of Non-propagative, Circulative Transmission

CV (internal) viruses are carried in the interior of the vector body (Gray and Gildow, 2003; Hogenhout *et al.*, 2008; Gray *et al.*, 2014). Some of the CV viruses propagate in the insect and are therefore termed CV-propagative. A list of CV and CV-propagative viruses is given in **Table 1**. The luteoviruses and the enamovirus PEMV are the best-studied CV viruses.

The transmission cycle

The transmission cycle of a CV virus includes six stages: (1) The aphid stylets, while piercing and sucking are inserted intercellularly to reach the phloem sieve elements, (2) ingestion from the infected host plant reaches the alimentary system of the vector, (3) passage of the virus through the vector's gut, (4) retention in the haemocoel or other internal tissues, (5) passage of the virus to the salivary glands, then, (6) via the salivary duct in the maxillary stylets, the virus is transported by the saliva to internal plant tissue (mostly phloem) (**Figure 2**).

Virus particles are retained in the haemolymph for several weeks. Survival in the haemolymph may depend on the presence of symbionin (see the following discussion). In the Luteoviridae, virus particles that are carried in the haemolymph need to cross the basal lamina of the accessory salivary gland (ASG) in order to be ejected by the salivary secretions to the plant tissues. The basal lamina of ASG consists of collagen that may serve as a selective filter, allowing differential binding and passage virus particles. On the way to exterior, virus particles must be transported across a third preferential barrier, the plasmalemma of the ASG, by receptor-mediated endocytosis. It is likely that the virus movement across these barriers is involved with different viral proteins or protein domains. Nanoviruses and begomoviruses, however, are specifically retained in the principal salivary glands (PSGs) (Watanabe and co-workers, cited in the study by Gray *et al.*, 2014). Circulative viruses are not transovarially transmitted with the exception of the begomovirus *Tomato yellow leaf curl virus*-Is (TYLCV-Is) that can also be sexually transmitted.

The role of viral capsid protein for insect transmission of circulative viruses

Protein subunits are important for the specificity of transmission of CV viruses. Rochow showed that strains of *Barley yellow dwarf virus* (BYDV) that are transmitted by one aphid species become transmitted by another aphid species if co-infected with another strain of BYDV (reviewed by Gray *et al.*, 2014). This

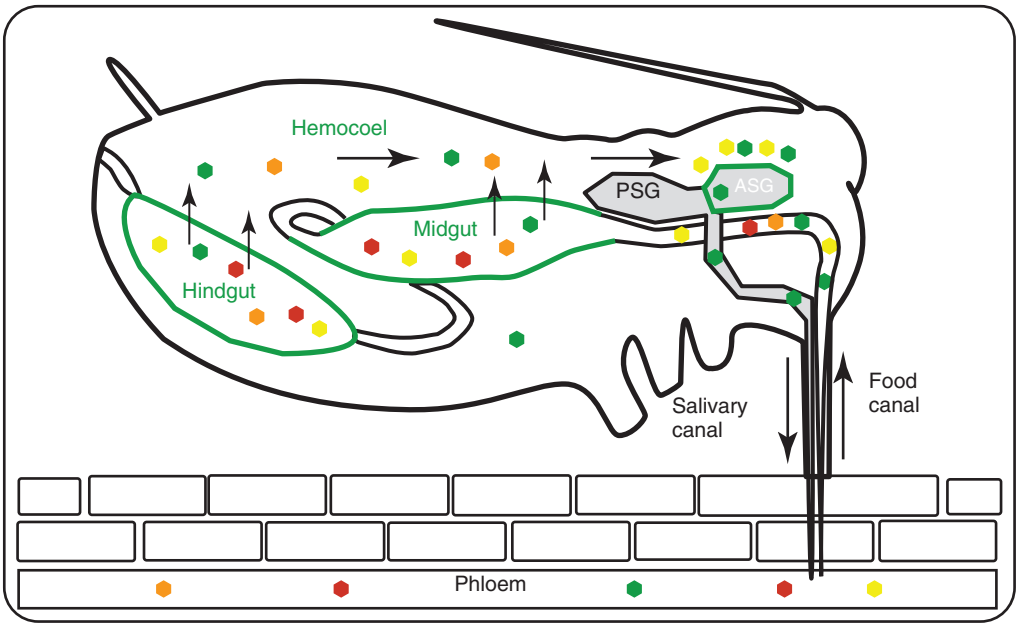

Figure 2 Schematic diagram of an aphid feeding and luteovirus transmission. Arrows indicate the circulative route for virus transmission through the insect's body. Virus particles circulate up through the food canal and cross the midgut and hindgut reaching the haemocoel. Then virus particles cross the accessory salivary glands and return to the plant via the salivary canal (from Gray *et al.*, 2014). Reproduced with permission form from Gray *et al.*, 2014 © Elsevier.

phenomenon was attributed by Rochow to heteroencaspsidation, where the non-transmissible RNA is encapsidated with some protein subunits of the transmissible strain.

Viral Proteins Involved in Transmission: the Coat Protein and the Read-Through Protein

Pea enation mosaic virus (PEMV) and luteovirus particles are composed of two types of capsomeres (Gray *et al.*, 2014). The predominant one is CP (*c*. 22–24 kDa). Another minor one, believed to be on the surface of the virion, is the read-through (RT) protein (*c*. 55–58 kDa). The RT protein results from a larger protein translated via the weak stop codon of the CP. The open reading frame encodes for a 72- to 74-kDa protein, of which the *C*-terminal half of the resulting protein is digested yielding a 55- to 58-kDa proteins. This protein is also found when CP is obtained from virus preparations. Virions encapsidated with the CP alone were not transmitted by aphids (but are found in the haemocoel following feeding). Moreover, these virions are infective when agro-inoculated (cited in the study by Gray and Gildow, 2003). These findings led to the conclusion that the RT protein is needed for aphid transmission. Mutants of *Beet western yellow virus* (BWYV) without the RT protein were not detectable in the ASGs and are non-transmissible by aphids. Mutants of RTD in various domains at the *C*-terminus did not affect aphid transmissibility. Mutation at the *N*-terminus of the RTD resulted in a protein that did not incorporate in the virus particle, but ingested particles are found in haemolymph. This suggests that the CP provides the signal for crossing the hindgut barrier, whereas the RT is the protein that associates with the ASG. However, recent reports show that particles encapsidated with the 22-kDa CP alone were found not only in the haemolymph but also in the ASG cells and in the salivary duct. This finding seems to be in contrast with the hypothesis that the RT is needed for crossing the ASG barrier.

In addition, in the case of the nanovirus *Faba bean necrotic yellows virus* (FBNYV), a helper protein is required for transmission. However, the origin – virus or plant – of the helper protein was yet not determined (Franz and co-workers as cited in the study by Gray *et al.*, 2014). Furthermore, proteins present in the phloem of cucurbits have been reported to enhance virus transmission of luteoviruses (Bencharki *et al.* and co-workers as cited in the study by Gray *et al.*, 2014).

Geminiviruses

The role of the CP in Geminivirus transmission was determined by exchanging the CP gene of two viruses differing in vector specificity. Thus, injection of the recombinant whitefly-borne *African cassava mosaic virus* (ACMV) with the *Beet curly top virus* (BCTV) CP enabled transmission by leafhoppers of ACMV. This suggests that the CP is needed to pass from the haemocoel to the salivary glands (Hull, 1994).

Insect Proteins Involved in Virus–Vector Interactions

Recently, the retention sites and specific proteins acting as receptors of both NC and CV viruses have been identified. A non-glycosylated protein deeply embedded in the chitin matrix of the aphid's maxillary stylets is involved in the retention of CaMV. This protein receptor present in three effective vector species but absent in a non-vector is located exclusively at the stylet tips in the bottom bed of the common duct where the food and salivary canals fuse together (Uzest *et al.*, 2007). The acrostyle, a specific anatomical structure within the common duct of aphid's maxillary stylets, was found to be the precise location where CaMV is retained by their vectors (Uzest *et al.*, 2010). Using a proteomic approach, four cuticular proteins that were extracted, separated and identified from *M. persicae* were able to bind *in vitro* to active potyviral HC-Pro but not to the mutated HC-Pro of the same virues (Dombrovsky *et al.*, 2007).

A similar approach was taken to show that four proteins from *Schyzaphis graminum* are involved in the ability to bind to the CV *Cereal yellow dwarf virus*-RPV polerovirus (Yang *et al.*, 2008). These proteins from *S. graminum* origin seem to play a key role in the high level of vector specificity, possibly by facilitating the passage of the virus through the gut and salivary gland tissues. Similarly, two proteins isolated from head tissues of the aphid vector, *Sitobion avenae*, have been identified as potential receptors for another CV virus (BYDV-MAV; *Luteoviridae*) (as cited in the study by Yang *et al.*, 2008).

Specific retention of a crinivirusin the anterior foregut and/or cibarium of its whitefly vector was mediated by the minor capsid protein CPm (Chen *et al.*, 2011). This was observed using a unique immunofluorescent localization approach in which virions or recombinant virus capsid components were ingested by whiteflies using artificial membrane feeding.

Transcriptional response of *B. tabaci* to a begomovirus identified 1606 genes and 157 biochemical pathways that were differentially expressed in viruliferous whiteflies, explaining why a negative impact of the virus on the longevity and fecundity of the B biotype of *B. tabaci* was found (reviewed by Gray *et al.*, 2014).

These findings may lead in the future to the use of viral genes encoding for proteins that are defective in the ability to assist transmission in transgenic plants. This may prevent vector inoculation. In addition, plants encoding for molecules (e.g. peptides) able to bind to cuticle protein receptors in the vector mouthparts may interfere with the process of virus retention. If successful, this form of virus prevention will complement those based on reduced multiplication and movement.

The Interaction between Bacterial Endosymbinots Proteins and Circulative Viruses

Aphids are known to host primary endosymbiotic bacteria of the genus *Buchnera* in specialised cells located in the abdomen mycetome (Gray *et al.*, 2014). These bacteria produce a protein called

symbionin. The RT protein mentioned earlier was found to interact with the GroEL (a bacterial protein showing homology with symbionin). Mutational analysis of the RT protein of beet western yellows luteovirus attributes the virus-binding capacity to a conserved region in the GroEL molecule. BWYV engineered to be encapsidated with CP alone (with no RT protein subunits) did not bind to *Buchnera* GroEL. In addition, *in vivo* studies showed that BWYV virions lacking the RT protein were significantly less persistent in the haemolymph than were virions with the RT protein. This led to the hypothesis is that the interaction between *Buchnera* GroEL and the RT protein protects the virus from rapid degradation in the haemolymph. Comparison of the RT domain from different luteoviruses and PEMV revealed several conserved amino acid residues that may be important for the interaction with *Buchnera* GroEL. In a more recent study, Hogenhout and co-workers demonstrated by mutational analysis of the gene encoding for *MpB* GroEL that the PLRV binding site is located in the equatorial domain and not in the apical domain of the symbionin (cited in the study by Gray and Gildow, 2003). The exact function of the symbionin, however, is not known. Bouvaine *et al.* (2011) reported that symbionin is restricted to the bacteriocytes and embryos, and not present in haemolymph or gut in *Acyrthosiphon pisum* and *Rhopalosiphum padi*, and therefore cannot participate in the protection of virus particles in the haemolymph. Therefore, it is not known if symbionin contributes in protecting luteoviruses as they travel through the haemolymph to the salivary gland or facilitate passage across the ASG barrier.

In addition to the primary endosymbiont *Portiera*, the whitefly *B. tabaci* carries a secondary endosymbiont, *Hamiltonella*, able to produce a GroEL protein that facilitates transmission of begomoviruses. Furthermore, some other whitefly endosymbionts of the *Rickettsia* genus contribute to the susceptibility to chemical insecticides and heat tolerance (reviewed by Gray *et al.*, 2014).

Analysis of Virus Transmission by Electrical Penetration Graphs (EPGs)

Electronic devices can distinguish between the intercellular and intracellular environments, which makes it possible to know when plant cell membranes are punctured by insect stylets (Fereres and Moreno, 2009; Stafford *et al.*, 2012). When a cell membrane is punctured a very distinctive electrical penetration graph (EPG), signal is recorded in the form of a potential drop (pd), which is associated to NP virus transmission (Powell, 1991). Other distinct waveforms and activities that are relevant to virus transmission of phloem-restricted viruses by aphids and whiteflies are E1 (phloem salivation, linked to virus inoculation) and E2 (phloem ingestion, linked to virus acquisition) (**Figure 3**).

Acquisition of stylet-borne viruses occurs after very brief (<1 min) probes and only when cell membranes are punctured by the stylets as shown by electron microscopy and EPG (pd signals). Detailed analysis of direct current-EPG signals during intracellular stylet punctures (pd) allows to differentiate three specific and distinct subphases: II-1, II-2 and II-3. Acquisition of stylet-borne viruses is associated to subphase II-3. Acquisition during the first pd is not only restricts to typical NP viruses such as CMV or

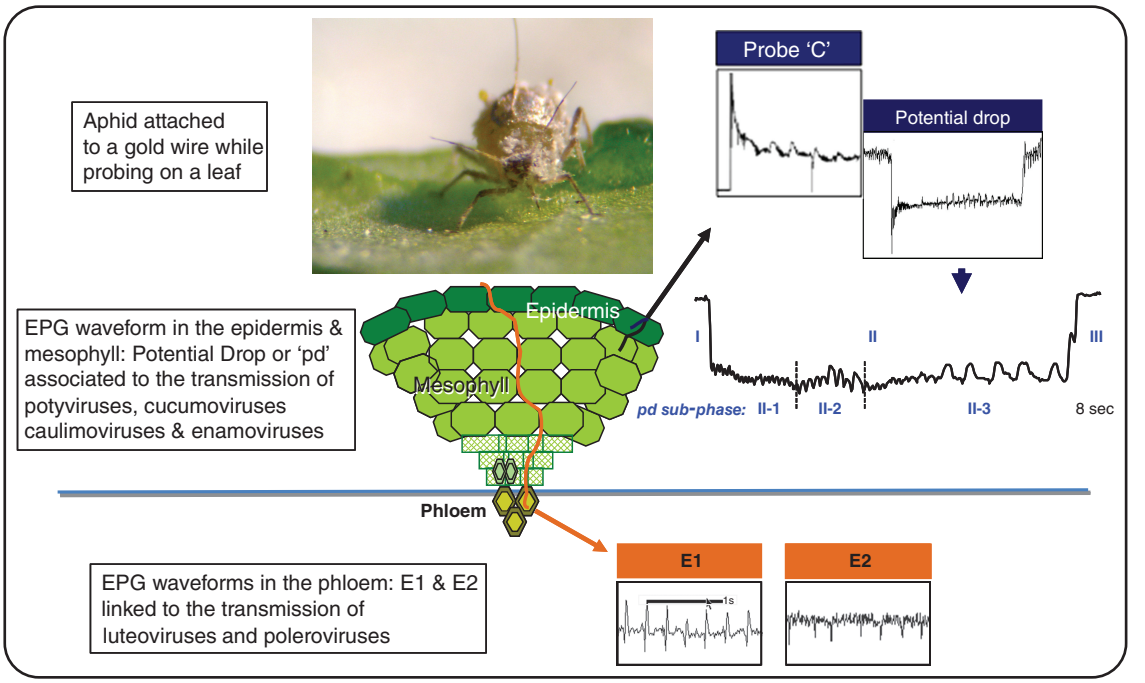

Figure 3 Electrical penetration graphs (EPGs) associated to the transmission of plant viruses by aphids.

PVY but also occurs for SP viruses such as CaMV. The main difference is that CaMV is preferentially acquired after committed phloem ingestion, whereas typical NP viruses are only acquired during brief superficial intracellular punctures. Work conducted by Fereres and co-workers showed that subphase II-1 within the first intracellular puncture was associated to the inoculation of NP viruses (PVY and CMV). On the basis of this finding and the fact that both salivary and alimentary canals fuse together in a common duct at the very tip of the maxillary stylets, the ingestion–salivation hypothesis was proposed. The results obtained also suggested that watery salivation was the mechanism involved in flushing out virus particles from the common duct during cell penetration. Later work using PEMV as a marker for intracellular salivation confirmed this hypothesis (Powell, reviewed by Fereres and Moreno, 2009). Later, EPG-assisted transmission studies showed that inoculation of the SP CaMV was linked exclusively to subphase II-2 of the first pd suggesting that NP and SP viruses are inoculated in a different manner (Moreno *et al.*, 2012). Studies on the feeding behaviour activities associated to the transmission of plant viruses by aphids, whiteflies, hoppers, mealybugs and thrips have been recently reviewed by Stafford *et al.* (2012).

Effects of Virus Infection on Vector Behaviour

As explained earlier, viruses need their vectors for survival and spread (Mauck *et al.*, 2012). Viruses have evolved and adapted to their insect vectors to increase their chances of transmission from plant to plant. There are many cases in which both viruses and vectors benefit from their mutual association by changes induced in their shared host plant after virus infection. In several cases, the intrinsic rate of increase and the proportion of alate aphid morphs often increase in virus-infected plants (reviewed by Fereres and Moreno, 2009). However, a more striking hypothesis (called vector manipulation) was proposed by Mauck and co-workers to explain how viruses could change vector behaviour in a way that the transmission and spread of a NC virus was enhanced. In their studies, they found that CMV-infected plants emitted a deceptive signal of plant volatiles that attracted aphids, which rejected the less suitable infected plant soon after probing. This particular pull–push behaviour of aphids is known to optimise NP virus transmission. Their findings show that the transmission mechanism is a major factor shaping pathogen-induced changes and how viruses have learned to manipulate vector behaviour to optimise their own spread. Similar results were found by Carmo-Sousa *et al.* (2014) that revealed a sharp change in aphid settling and probing behaviour over time when exposed to CMV-infected plants. Soon after, plant contact aphid vectors dramatically increased the number of short superficial probes and intracellular punctures when exposed to CMV-infected plants. At a later stage (second hour of recording), aphids diminished their feeding on CMV-infected plants as indicated by much less time spent in phloem salivation and ingestion (E1 and E2). Such changes in aphid behaviour on CMV-infected plants leads to optimum transmission and spread of the virus.

Aphid attraction to volatiles emitted from plants infected with CV viruses was also reported (Eigenbrode *et al.*, 2002). Furthermore, attraction to infected plants may be reversed after the vector feeds on a virus source and becomes viruliferous. Rajabaskar *et al.* (2013) found that non-viruliferous *M. persicae* settled preferentially on potato plants infected with *Potato leafroll virus*, whereas the reverse was the case for viruliferous aphids that preferred mock-inoculated plants.

Many other examples show how plant viruses can manipulate vector behaviour to enhance their transmission and spread. TSWV-infected thrips changed their probing behaviour after virus acquisition and made much more inoculative probes than non-infected thrips (Stafford *et al.*, 2011). The settling and feeding behaviour of *B. tabaci* was modified after acquisition of TYLCV-Is in a way that whiteflies settled faster and increased the duration of the salivation phase linked to the transmission of the virus (Moreno-Delafuente *et al.*, 2013).

Control of Virus Diseases by Interfering with Vectors and Transmission

In this article, we discuss measures aimed against vector activity and transmission (Antignus, 2012; Bragard *et al.*, 2013). These measures are among the most successful approaches used to suppress virus epidemics. Other control measures (e.g. breeding for resistance to the pathogen, sanitation, prevention and natural and pathogen-derived resistance) will not be discussed in this article and reader should consult the Further Reading list. Control measures against vectors and vector activities can be grouped into four classes: (1) reducing vector populations, (2) reducing virus sources, (3) interference with vector landing and (4) interference with the transmission process.

Reducing vector populations: Despite the wide range of insecticides available, chemical control is not the preferred solution to prevent vector activity. Many viruses are introduced into crops by visiting insects that inoculate during their first probing activities. Vectors for NP (and partly SP) viruses need relatively short inoculation times – much shorter than the time needed for insecticides to kill. In addition, insecticides can induce restlessness in insects, with the result that they make more inoculation attempts than do calm insects. Exceptions are vectors that colonise the crop and transmit phloem-restricted viruses, for which insecticides may reduce virus spread. New biotechnology-based approaches such as genetically modified aphid-resistant plants that express protease inhibitors, dsRNA, antimicrobial peptides or repellents can effectively reduce vector numbers (Will and Vilcinskas, 2013). In aphids, RNAi-mediated gene silencing can reduce the expression of salivary gland proteins or kill the pea aphid, *A. pisum* when fed species-specific dsRNA targeting *vATPase* transcripts (Whyard *et al.*, 2009).

Reducing virus sources: Use of virus-free seeds and/or propagative organs results in minimal primary infection. This can be complemented by removal of sources of infection in and around the crop, removal of plant remains from a former season and, if necessary, creation of a time gap between crops and/or space gap

Figure 4 Non-woven agrotextiles are commonly used as a physical barrier to protect vegetables from insect landing and preventing virus epidemics.

between plots. These operations will reduce the numbers of viruliferous insects that reach the crop.

Interference with vector landing on crops is achieved by altering the attraction of insects to colours. Insects (e.g. aphids) are repelled from reflective surfaces: this effect led to the use of metallic reflective surfaces, straw mulches or kaolin particle films. Landing can be prevented by use of physical barriers (**Figure 4**). Insect-proof nets greatly reduce virus incidence and the need for insecticide applications against begomoviruses in tomato. Camouflaging nets greatly reduce insect landing and also virus infection. This measure is now being used commercially for the protection of papaya from *Papaya ring spot virus* in Taiwan.

One of the most effective ways to interfere with vector landing and navigation is the use of UV-absorbing plastics and nets. Polyethylene sheets and nets that absorb UV-light interfere with insect vision, and consequently, were found to greatly reduce virus incidence (**Figure 5**). An impressive reduction of insect landing was recorded for whiteflies, aphids or thrips. The mode of action and benefits of using this type of materials has been reviewed by Antignus 2012.

Interference with the transmission process: Mineral oils are hydrophobic substances that interfere with virus acquisition and retention by aphids. Mineral oil of an appropriate viscosity and unsulfonated residues was found effective to reduce the efficiency of transmission by vectors. This measure is still popular for protection against NP viruses, particularly in nurseries. The mode of action seems to be by interference with virus binding by probing aphids. The leaf surface must be fully covered: full coverage demands frequent applications (up to twice a week) of large volume at high pressure. Combination of oil with pyrethroids (insecticides that have insect-repelling qualities) was tested successfully in Israel and in England (Raccah, 1986). Novel molecules – for example, peptides – could be designed to outcompete with virus CP or non-structural virus-encoded proteins needed for virus attachment to insect receptors and thus interfere with transmission (Blanc *et al.*, 2014).

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Figure 5 Tomato plants grown under UV-absorbing nets (a) protect from TYL. The control plot at the (b) (standard net) shows tomato plants with severe symptoms.

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