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A comprehensive review on transmission mechanism of plant viruses by insects

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Abstract
Most plant viruses depend on insect vectors for their survival, transmission, and spread. These viruses are transmitted by insects through two principal modes: circulative and non-circulative. In the circulative mode, the virus circulates through the insect's haemocoel (CV), whereas in the non-circulative mode, the virus is carried on the cuticle lining of the insect's mouthparts or foregut (NC). The transmissibility and specificity between non-circulative viruses and their vectors depend on the coat protein (CP) of the virus, in addition to virus-encoded helper proteins. Circulative viruses cross the insect's gut, circulate in the haemocoel, and eventually cross the salivary glands to render the insect infective. For circulative luteoviruses, small coat proteins and the read-through protein (RTD) are essential for transmission. Electrical penetration graphs have provided evidence on insect feeding behavior and virus transmission. Recent studies have shown that viruses can modify vector behavior to enhance transmission. Cultural, physical, and novel biotechnological tools can provide virus control by interfering with vector landing and the retention of viruses in their vectors.

Keywords: Vector, virus, transmission, potyvirus, Caulimovirus, Geminivirus, Potexvirus

Introduction
Insect vectors of plant viruses are found in 7 of the 32 orders of the class Insecta, with Hemipterans being the most significant, comprising over 70% of all known insect-borne viruses. Among these, aphids and whiteflies are the primary vectors, transmitting more than 500 virus species. Viruses are classified based on how long the vector remains viruliferous - persistent, semi-persistent (SP), or non-persistent (NP) - or by the route the virus takes within its vector - non-circulative (NC) or circulative (CV). More recently, a third classification has been proposed, based on the localization of virus-vector retention sites: cuticula-borne or salivary gland-borne. Various viral and insect proteins control some virus-vector associations, but many remain unknown. Interference with vector landing by manipulating insect vision, along with novel molecules that outcompete viruses from their retention sites in vectors, could help reduce plant virus epidemics.

Importance of insect as a vector
Most plant viruses rely on vectors for their survival due to two principal reasons.

1. An impermeable cuticle coats the plant epidermis, preventing the entry of virus particles, unlike animal viruses that can readily enter through natural openings. Most vectors are insects, although non-insect vectors include mites, nematodes, and fungi. Several plant viruses may also spread by contact or vegetative reproduction. Many insects, such as hemipterans, are well adapted to their role as vectors due to their ability to pierce the epidermis and delicately deposit the virus in the cytoplasm without compromising the integrity of the plant cell. Recent findings suggest that viruses have adapted to their vectors by modifying their behavior to maximize their own spread.
2. Plants are rooted and lack independent mobility, so many viruses rely on insects for transport among hosts. Unlike animals, which can move independently and carry viruses to new niches, plants depend on insects to facilitate the spread of viral infections.

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

The primary transmission modes: Persistent versus Non-persistent; Circulative versus Non-circulative

Plant viruses exhibit a high degree of specificity towards the groups of insects capable of transmitting them; a virus transmissible by one type of vector typically cannot be transmitted by another. This article excludes circulative (CV) viruses that replicate within their insect vectors. A summary of the principal insect-borne virus groups and their respective vectors can be found in Table 1.

Modes of transmission

In the 1930s, Watson and Roberts proposed different modes of virus transmission by insects, based on how long the virus is retained by the vector. Initially, they identified two modes: non-persistent (NP) for short retention, defined as less than the time the virus survives in leaf extracts, and persistent for extended retention, often lasting the lifetime of the vector. However, they found that several viruses exhibited intermediate retention periods in their vectors. This led Sylvester to designate the term SP viruses [1]. Over 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, while persistent viruses were termed circulative (CV). Additional attributes were eventually attached to each of these modes of transmission. 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 those not colonizing the crop. SP viruses need longer periods (hours) for acquisition and transmission compared to NP viruses and have a narrower range of vector species. However, they do not require a latent period and are lost when the vector molts. Persistent viruses require several hours or even days for efficient acquisition and inoculation. They have a narrow range of vectors, mostly those that colonize the crop, pass through molts, and need a latent period.

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

Mechanism of non-persistent transmission

Virus particles, rather than their naked nucleic acids, are the pathogenic units transmitted by insects to initiate infection (2). On the other hand, viral nucleic acids (either DNA or RNA) are sufficient to cause infection when introduced to plant cells by artificial means, such as rubbing or bombardment and agro-infections.

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

Virus group	Mode	Persistence	Localization	Vector involved
Alfamovirus	NP	Few hours	Stylets	Aphids
Badnavirus	S	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, P- Persistent, SP- Semi-persistent.

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

Feature	Non-persistent	Semi-persistent	Persistent circulative
Duration of retention	Brief (few hours)	Intermedia (few days)	Long (days to months)
Duration of acquisition and transmission	Brief (Seconds)	Intermediate (hours)	Long
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

All circulative viruses, except for Pea Enation Mosaic Virus (PEMV), are transmitted in a persistent manner. PEMV is unique in that it is assisted by an umbravirus, which enables it to invade tissues beyond the phloem. Additionally, the duration of the acquisition and inoculation periods for PEMV is similar to that of viruses transmitted in a non-persistent manner.

This indicates that protein molecules encapsulating nucleic acid play a crucial role in interacting with specific sites present in the vector. The investigation into the role of the coat protein (CP) in virus transmissibility has been facilitated by the existence of virus strains differing in their affinity for vector species, as well as strains that lose transmissibility following continuous mechanical inoculation (further details in subsequent sections). Recently, the precise location and chemical composition of the initial NC virus receptor within vector mouthparts have been pinpointed. Furthermore, advancements in electronic devices have aided in elucidating the specific probing behaviors of insect vectors associated with the transmission of plant viruses.

The role of the capsid protein in the transmission of non-persistent viruses.

Cucumovirus

Gera and colleagues demonstrated that the genome of a poorly transmissible strain of Cucumber mosaic virus (CMV) became transmissible when encapsulated in vitro with the capsid protein (CP) of a highly transmissible strain [4-6]. Subsequent studies by Perry and colleagues involved designing chimeric RNA 3 cDNA constructs to introduce mutations into the capsid protein (CP) (4). As a result of these investigations, researchers identified three amino acid mutations in the capsid protein (CP) that influenced the transmission of CMV by *Aphis gossypii*. In a subsequent study, they found that CMV transmissibility by *Myzus persicae* required two additional mutations in the CP, specifically at positions 25 and 214, in addition to those previously identified at positions 129, 162, and 168 [4]. Changes in charge within the metal-ion-binding βH–βI loop, which is exposed on the surface of certain non-transmissible CMV mutants, are believed to disrupt the interaction between the virus and its vector [7].

Potyviruses

To identify the determinants of potyvirus transmission by aphids, researchers compared the amino acid sequences of the coat protein (CP) from aphid-transmissible (AT) and non-aphid-transmissible (NAT) virus strains. They discovered a conserved amino acid triplet, Asp-Ala-Gly (DAG), located within the highly variable and exposed amino terminal end of the CP. NAT strains exhibited a mutated triplet, typically Gly mutated to Glu (DAG to DAE), which resulted in the loss of transmissibility in an AT strain of Tobacco vein mottling virus (TVMV). The importance of the DAG motif in aphid transmission was further confirmed in an NAT strain of Zucchini yellow mosaic virus (ZYMV), where changing Thr to Ala (DTG to DAG) restored transmissibility. Additionally, alterations in amino acids near the DAG triplet were also found to affect transmission of TVMV [5]. Electron microscopy studies revealed that the DAG motif in potyviruses plays a role in retaining the virus within the aphid's mouthparts. This mechanism likely occurs through interaction between DAG and a virus-encoded protein called the helper component (HC), as demonstrated using protein-blotting overlay techniques [5].

Potexvirus

Potato aucuba mosaic virus (PAMV) cannot be transmitted by aphids on its own, but it can be transmitted with the assistance of potyviruses. The DAG motif within the coat protein (CP) sequence of PAMV is absent in *Potato virus X* (PVX). However, when the DAG motif from PAMV was transferred to PVX, PVX gained the ability to be transmitted by aphids [5].

Potyviruses

Kassanis and Govier first reported the helper phenomenon [5]. They initially demonstrated that the non-aphid-transmissible (NAT) virus Potato aucuba mosaic virus (PAMV) could be transmitted in the presence of the aphid-transmissible Potato virus Y (PVY). Subsequently, they established that potyvirus transmission requires a helper component (HC) in addition to viral particles. Moreover, they found that transmission occurs only when the virus is acquired alongside or after the acquisition of the HC. This observation led to the development of the 'bridge' hypothesis, suggesting that the HC binds to aphid mouthparts on one side and to virions on the other, thereby ensuring virus retention until release into the next host. Sequencing of the potyviral genome and identification of the resulting protein characterized it as a non-structural protein encoded by the HC-Pro region of the potyvirus genome. The helper function in transmission was localized to the N-terminal and central regions of HC-Pro. HC proteins have a predicted molecular mass ranging from 50 to 60 kDa, with the proposed biologically active form being a dimer. By comparing strains with active and inactive HC, domains crucial for vector transmission were identified. For Tobacco vein mottling virus (TVMV), loss of HC activity correlated 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 found in other potyviruses (such as mutants of PVY and Zucchini yellow mosaic virus [ZYMV] HCs). Importantly, the KITC motif of HC was not implicated in virion binding, as demonstrated by efficient binding of transmission-defective ZYMV-Ct with K instead of E in the KLSC motif in overlay blotting experiments [5]. In the central region of the HC-Pro gene, another conserved motif, Pro-Thr-Lys (PTK), was identified as crucial for assisting in the transmission of *Zucchini yellow mosaic virus* (ZYMV). A mutation from Pro to Ala within the PTK motif led to the loss of helper activity. Furthermore, the PTK motif was shown to influence the binding of HC to virions in overlay blotting

experiments [5].

The function of the HC in retaining the virus in the stylet was demonstrated by comparing aphids that fed on mixtures containing transmissible Tobacco etch virus (TEV) or *Tobacco vein mottling virus* (TVMV) virions alongside functional *Potato virus Y* (PVY) HC or TVMV HC (with the KITC motif), versus those that fed on non-functional HC (with the EITC motif) [5].

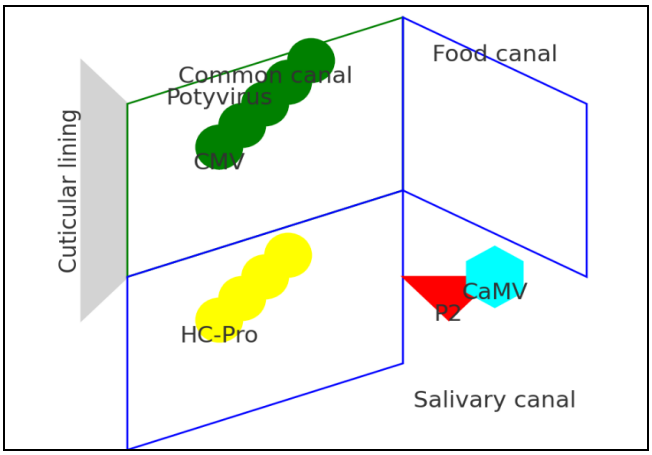

Fig 1: The model describes various strategies for virus–vector interactions in non-circulative transmission by aphids

These strategies facilitate the retention of virus particles on the common canal of the maxillary stylets at the surface of the cuticular lining. In the capsid strategy, exemplified by 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 forms a 'molecular bridge' between the virus and the vector. HC-Pro can be acquired either alone or in conjunction with the virion. Caulimoviruses (CaMV) also employ the helper strategy, but they use a different protein (P2) to act as a bridge between the virus and the vector.

Caulimovirus

Caulimoviruses have also adopted a helper-dependent transmission strategy, but in a more complex manner compared to potyviruses. Cauliflower mosaic virus (CaMV) requires two viral-encoded non-structural proteins, P2 and P3. A P2-P3-virion complex is formed, where P2 binds to the aphid while P3 binds to the virions [8]. Furthermore, the HC motif directly involved in specific vector recognition was identified at position 6 of the N-terminus of P2. A single amino acid mutation, which can occur spontaneously, alters the spectrum of vectors capable of transmitting CaMV [9, 10] 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 components are involved in several other transmission systems. For instance, the semi-persistently transmitted Parsnip yellow fleck virus is not transmissible by aphids unless acquired together with the Anthriscus yellows virus. A dense material with virus-like particles was observed in aphids' mouthparts after acquiring the virus. Rice tungro spherical virus (RTSV) is transmissible by several *Nephottetix* leafhopper species and aids in the transmission of a second virus, the Rice Tungro bacilliform virus. Additionally, Maize chlorotic dwarf virus is semi-persistently transmitted by leafhoppers and is believed to have helper components [11]. The lack of vector transmissibility of purified virions led to the speculation that a helper component is needed for the transmission of carlaviruses and closteroviruses.

Modes of Transmission of Plant virus by Beetle

Beetle vectors of plant viruses are known in four families: *Chrysomelidae, Coccinellidae, Curculionidae,* and *Meloidae* [12]. Beetle-borne viruses have a unique mode of transmission, being carried in the beetle's regurgitant with no latent period in the vector. Initially, it was assumed that components in the regurgitant selectively inactivated particles of non-transmissible viruses. However, mixing various viruses with beetle regurgitant had an insignificant effect on most viruses, whether beetle-borne or not. Some beetle-borne viruses are circulative (CV), as they were found to move into the insect's haemolymph immediately after ingestion. Beetles can also become viruliferous by injecting the virus into their haemolymph. Interestingly, research by Wang and colleagues found that beetles might transmit viruses even if the viruses are not present in the haemolymph. The retention of infectivity varies among beetle vectors; for example, *Epilachna varivestis* retains *Cowpea severe mosaic virus* for one day, whereas *Cerotoma trifurcata* can transmit the same virus for several days. The virus does not propagate in the beetle, as the virus titer declines over time. Gergerich and colleagues [12] demonstrated the unique role of regurgitant in the infection process. While viruses not transmissible by beetles were mechanically infectious to wounded hosts, only beetle-borne viruses remained infectious when regurgitant was added to the inoculum mixture. The inability of virus particles to infect hosts was not due to inactivation since purified virus particles regained infectivity once separated from the regurgitant. This finding suggests that an inhibitor in the regurgitant affects the host or the interaction between the virus and host. Beetle-transmissible viruses differ from other viruses in their rapid translocation to non-wounded cells through the xylem and the way they initiate primary infection.

The mechanism of Persistent non-propagative type Transmission

In this mechanism viruses are carried inside the vector body for transmission [13-15]. Some circulative viruses (CV) propagate within the insect and are therefore termed CV-propagative. A list of CV and CV-propagative viruses is provided in Table 1. The luteoviruses and the Enamovirus PEMV are among the best-studied CV viruses.

Transmission process

The transmission process of a circulative virus (CV) includes six stages: (1) The aphid inserts its stylets intercellularly while piercing and sucking to reach the phloem sieve elements, (2) ingestion from the infected host plant enters the vector's alimentary system, (3) the virus passes through the vector's gut, (4) the virus is retained in the haemocoel or other internal tissues, (5) the virus passes to the salivary glands, and (6) the virus is transported by saliva through the salivary duct in the maxillary stylets to the plant's internal tissues, primarily the phloem.

Virus particles are retained in the haemolymph for several weeks, with their survival potentially depending on the presence of symbionin. In the Luteoviridae family, virus particles carried in the haemolymph need to cross the basal lamina of the accessory salivary gland (ASG) to be expelled into plant tissues via salivary secretions. The basal lamina of the ASG, composed of collagen, may serve as a selective filter, allowing differential

binding and passage of virus particles. During their journey to the exterior, virus particles must traverse a third barrier, the plasmalemma of the ASG, via receptor-mediated endocytosis. This movement across barriers likely involves different viral proteins or protein domains. In contrast, nanoviruses and begomoviruses are specifically retained in the principal salivary glands (PSGs). Circulative viruses are not transovarially transmitted, with the exception of the begomovirus Tomato yellow leaf curl virus-Is (TYLCV-Is), which can also be sexually transmitted.

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

Luteovirus and *Pea enation mosaic virus* particles are made by two types of Capsomeres [15]. The predominant viral protein is the coat protein (CP), which is approximately 22–24 kDa. Another, more minor protein believed to be on the surface of the virion is the read-through (RT) protein, which is about 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 a 72- to 74-kDa protein, of which the C-terminal half is digested, yielding the 55- to 58-kDa RT proteins. This protein is also found when CP is obtained from virus preparations. Virions encapsidated with the CP alone are not transmitted by aphids, although they are found in the haemocoel following feeding. Moreover, these virions remain infective when agro inoculated [13]. These findings led to the conclusion that the RT protein is necessary for aphid transmission. Mutants of Beet western yellow virus (BWYV) lacking the RT protein were not detectable in the accessory salivary glands (ASGs) and were non-transmissible by aphids. Mutations in the C-terminal domain of the read-through domain (RTD) did not affect aphid transmissibility. However, mutations at the N-terminus of the RTD resulted in a protein that did not incorporate into the virus particle, though ingested particles were found in the haemolymph. This suggests that the coat protein (CP) provides the signal for crossing the hindgut barrier, whereas the RT protein associates with the ASG. However, recent reports indicate that particles encapsidated with the 22-kDa CP alone were found not only in the haemolymph but also in the ASG cells and the salivary duct. This finding seems to contradict the hypothesis that the RT protein is necessary for crossing the ASG barrier.

In the case of the nanovirus Faba bean necrotic yellows virus (FBNYV), a helper protein is also required for transmission. However, the origin of this helper protein - whether from the virus or the plant - has not yet been determined [15]. Additionally, proteins present in the phloem of cucurbits have been reported to enhance the transmission of *luteoviruses* [15].

Geminiviruses

The role of the coat protein (CP) in Geminivirus transmission was elucidated by exchanging the CP gene between two viruses with different vector specificities. When the CP gene of Beet curly top virus (BCTV) was introduced into the whitefly-borne African cassava mosaic virus (ACMV), it enabled ACMV to be transmitted by leafhoppers. This suggests that the CP is essential for the virus to pass from the haemocoel to the salivary glands [11].

Vector proteins involved in virus-vector relationship

Recently, researchers have identified the retention sites and specific proteins acting as receptors for both non-circulative (NC) and circulative viruses (CV). For instance, a non-glycosylated protein, deeply embedded in the chitin matrix of the aphid's maxillary stylets, is involved in the retention of Cauliflower mosaic virus (CaMV). This protein receptor, present in three effective vector species but absent in a non-vector species, is located exclusively at the tips of the stylets in the bottom bed of the common duct where the food and salivary canals fuse together [16]. The acrostyle, a specific anatomical structure within the common duct of an aphid's maxillary stylets, has been identified as the precise location where Cauliflower mosaic virus (CaMV) is retained by its vectors [17]. Using a proteomic approach, four cuticular proteins extracted, separated, and identified from *Myzus persicae* were found to bind in vitro to active potyviral HC-Pro but not to the mutated HC-Pro of the same viruses [18].

A similar approach demonstrated that four proteins from *Schizaphis graminum* are involved in binding to the circulative virus (CV) Cereal yellow dwarf virus-RPV polerovirus [19]. These proteins from *Schizaphis graminum* appear 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 the head tissues of the aphid vector *Sitobion avenae* have been identified as potential receptors for another circulative virus, Barley yellow dwarf virus-MAV (BYDV-MAV; *Luteoviridae*) [19]. The specific retention of a crinivirus in the anterior foregut and/or cibarium of its whitefly vector is facilitated by the minor capsid protein CPm [20]. This observation was made using a distinctive immunofluorescent localization method, where virions or recombinant virus capsid components were ingested by whiteflies through artificial membrane feeding. The identification of 1606 genes and 157 biochemical pathways that were differentially expressed in viruliferous whiteflies by the transcriptional response of *B. tabaci* to a begomovirus explains why the virus had a detrimental effect on the longevity and fertility of the B biotype of *B. tabaci* [15]. These results might eventually lead to the adoption of viral genes that code for proteins that are faulty in their ability to aid in the transmission of viruses in transgenic plants. This could stop the inoculation of vectors. Furthermore, the process of viral retention may be hampered by plants that encode for compounds (such as peptides) that can bind to cuticle protein receptors in the vector mouthparts. If effective, this method of preventing viruses will support those that focus on limiting their mobility and proliferation.

Interaction between Circulative virus and Bacterial Symbiont Proteins

It is known that aphids harbour primary endosymbiotic bacteria belonging to the Buchnera genus in specific cells found in the mycetome of the abdomen [15]. These types of bacteria produce particular protein called symbionin. The RT protein was found to interact with GroEL, a bacterial protein homologous to symbionin. Mutational analysis of the RT protein in beet western yellows luteovirus (BWYV) revealed that its virus-binding capacity is attributed to a conserved region in the GroEL molecule. BWYV engineered to be encapsidated with coat protein (CP) alone, without RT protein subunits, did not bind to Buchnera GroEL. Additionally, in vivo studies showed that BWYV virions lacking the RT protein were significantly less persistent in the haemolymph compared to virions with the RT protein. This observation led to the hypothesis that the interaction between Buchnera GroEL and the RT protein protects the virus from rapid degradation in the haemolymph. A comparison of the RT domain from different luteoviruses and

pea enation mosaic virus (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 through mutational analysis of the gene encoding for MpB GroEL that the PLRV binding site is in the equatorial domain, not in the apical domain, of symbionin [13]. The particular function of symbionin is still unknown. Bouvaine reported that symbionin is confined to bacteriocytes and embryos in *Acyrthosiphon pisum and Rhopalosiphum padi*, and is absent in the haemolymph and gut. Consequently, symbionin cannot be involved in protecting virus particles in the haemolymph. It remains uncertain whether symbionin plays a role in safeguarding luteoviruses during their journey through the haemolymph to the salivary gland or in facilitating their passage across the ASG barrier [21]. In addition to the primary endosymbiont Portiera, the whitefly *Bemisia tabaci* harbors a secondary endosymbiont, Hamiltonella, which produces a GroEL protein facilitating the transmission of begomoviruses. Moreover, certain other endosymbionts of the Rickettsia genus in whiteflies contribute to susceptibility to chemical insecticides and heat tolerance [15].

Virus transmission analysis by Electrical Penetration Graphs (EPGs)

Because electronic instruments can differentiate between intracellular and intercellular environments, it is possible to determine whether insect stylets have breached plant cell membranes [22, 23]. When a cell membrane is punctured, a distinct electrical penetration graph (EPG) signal in the form of a potential drop (pd) is recorded, which is associated with non-persistent (NP) virus transmission [24]. Acquisition of stylet-borne viruses occurs after very brief probes (less than one minute) and only when cell membranes are punctured by the stylets, as shown by electron microscopy and electrical penetration graph (EPG) signals. Detailed analysis of direct current EPG signals during intracellular stylet punctures (pd) allows for differentiation into three specific and distinct subphases: II-1, II-2, and II-3. Acquisition of stylet-borne viruses is associated with subphase II-3. Acquisition during the first pd is not restricted to typical non-persistent (NP) viruses such as CMV or PVY but also occurs for semi-persistent (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 with the inoculation of NP viruses (PVY and CMV). Based on 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 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 [22]. Subsequently, EPG-assisted transmission investigations revealed that the SP CaMV infection was solely associated with subphase II-2 of the first PPD, indicating that the NP and SP viruses are injected differently [25]. Recent reviews have examined the feeding behavior activities linked to the transmission of plant viruses by aphids, whiteflies, hoppers, mealybugs, and thrips [23].

Changes occur in insect vector after virus infection

As discussed earlier, virus needs vector for survival and spread [26]. Viruses have become more likely to spread from one plant to another by evolving and adapting to their insect carriers. Numerous instances exist wherein both viruses and following virus infection, changes are brought about in their shared host plant, which benefits the vectors from their mutual connection. The fraction of alate aphid morphs and the intrinsic rate of increase in virus-infected plants both frequently rise in a few instances [22]. However, Mauck and colleagues proposed a more compelling hypothesis known as vector manipulation to explain how viruses can alter vector behavior to enhance the transmission and spread of non-circulative (NC) viruses. In their research, they discovered that CMV-infected plants emit plant volatile signals that attract aphids, which then quickly reject the less suitable infected plants after probing. This pull-push behaviour of aphids optimizes the transmission of non-persistent (NP) viruses. Their findings underscore how the transmission mechanism plays a crucial role in shaping pathogen-induced alterations, demonstrating how viruses have evolved to manipulate vector behaviour to maximize their own dissemination. Their studies revealed a notable shift in aphid settling and probing behaviour over time upon exposure to CMV-infected plants. Initially, aphid vectors significantly increased the number of short superficial probes and intracellular punctures. However, during a later stage (the second hour of recording), aphids reduced their feeding on CMV-infected plants, spending less time in phloem salivation and ingestion (E1 and E2). These changes in aphid behaviour on CMV-infected plants contribute to the optimal transmission and spread of the virus [27]. It has also been noted that aphids are drawn to the volatiles released by plants infected with CV viruses [28]. Furthermore, once the vector becomes viruliferous and feeds on a viral source, attraction to infected plants may reverse. In their studies Non-viruliferous *M. persicae* landed preferentially on potato plants infected with Potato Leafroll virus, according to research by Rajabaskar and colleagues, but viruliferous aphids preferred mock-inoculated plants [29]. Numerous more instances demonstrate how plant viruses can alter the behaviour of their vectors to improve their ability to propagate and proliferate.

After acquiring the virus, TSWV-infected thrips altered their probing habit and produced significantly more inoculative probes than uncontaminated thrips [30]. Following the acquisition of TYLCV-Is, *B. tabaci's* settling and feeding behaviour changed such that whiteflies settled more quickly and prolonged the salivation phase, which is associated to the transmission of the infection [31].

Management of Viral Diseases via Interfering with Vectors and Spread

These strategies represent some of the most effective approaches employed to mitigate virus epidemics. Other control methods, such as breeding for pathogen resistance, sanitation practices, and natural or pathogen-derived resistance, are not covered in this article but can be explored further in the suggested reading list. Measures targeting vectors and their activities can be categorized into four main classes: (1) decreasing vector populations, (2) minimizing virus reservoirs, (3) disrupting vector landing, and (4) interfering with the transmission process.

Vector population reduction

Despite the availability of a wide range of insecticides, chemical control is not the preferred method for preventing vector activity. Many viruses enter crops through insects that inoculate them during their initial probing activities. Vectors of non-

persistent (NP) and partially persistent (SP) viruses require relatively short inoculation times, much briefer than the time it takes for insecticides to take effect. Moreover, insecticides can agitate insects, leading to increased inoculation attempts compared to calm insects. However, there are exceptions with vectors that establish colonies within crops and transmit phloem-limited viruses, where insecticides may help reduce virus spread. Emerging biotechnology-based approaches, such as genetically modified aphid-resistant plants expressing protease inhibitors, double-stranded RNA (dsRNA), antimicrobial peptides, or repellents, show promise in effectively reducing vector populations [32]. In aphids, RNA interference (RNAi) can be used to decrease the expression of salivary gland proteins or induce mortality in the pea aphid, A. pisum, by feeding them species-specific double-stranded RNA (dsRNA) targeting vATPase transcripts [33].

Reduction of Virus Sources

The use of virus-free seeds and/or propagative organs minimizes initial infections. This strategy is enhanced by removing infection sources within and around the crop, clearing plant residues from previous seasons, and potentially implementing crop rotation or spatial gaps to deter aphid retention. Mineral oil with suitable viscosity and low unsulfonated residues has proven effective in reducing vector transmission efficiency, particularly for non-persistent (NP) viruses, commonly applied in nurseries. Its mechanism involves interfering with virus binding during aphid probing. Effective application requires full leaf coverage, necessitating frequent treatments (up to twice a week) with large volumes at high pressure. Combining oil with pyrethroids, insecticides with repellent properties, has shown successful results in trials conducted in Israel and England [34]. Innovative molecules, such as peptides, could be engineered to competitively inhibit virus coat proteins or non-structural virus-encoded proteins essential for virus attachment to insect receptors, thereby disrupting transmission [35].

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