Looking Through the Lens of 'Omics Technologies: Insights into the Transmission of Insect Vector-borne Plant Viruses

Jennifer R. Wilson[1,2], Stacy L. DeBlasio[1,2,3], Mariko M. Alexander[1,2] and Michelle Heck[1,2,3]*

[1]Plant Pathology and Plant Microbe Biology Section, School of Integrative Plant Sciences, Cornell University, Ithaca, NY, USA.
[2]Boyce Thompson Institute, Ithaca, NY, USA.
[3]Emerging Pests and Pathogens Research Unit, United States Department of Agriculture – Agricultural Research Service, Ithaca, NY, USA.

*Correspondence: mlc68@cornell.edu

https://doi.org/10.21775/cimb.034.113

Abstract

Insects in the orders Hemiptera and Thysanoptera transmit viruses and other pathogens associated with the most serious diseases of plants. Plant viruses transmitted by these insects target similar tissues, genes, and proteins within the insect to facilitate plant-to-plant transmission with some degree of specificity at the molecular level. 'Omics experiments are becoming increasingly important and practical for vector biologists to use towards better understanding the molecular mechanisms and biochemistry underlying transmission of these insect-borne diseases. These discoveries are being used to develop novel means to obstruct virus transmission into and between plants. In this chapter, we summarize 'omics technologies commonly applied in vector biology and the important discoveries that have been made using these methods, including virus and insect proteins involved in transmission, as well as the tri-trophic interactions involved in host and vector manipulation. Finally, we critically examine the limitations and new horizons in this area of research, including the role of endosymbionts and insect viruses in virus–vector interactions, and the development of novel control strategies.

Advances in whole genome sequencing of insect vectors of plant viruses fuels discovery

Major advances in next generation sequencing technologies and their increased affordability has led to an explosion in the availability of whole-genome sequence data for each biological player in the virus–vector–host relationship: virus, vector and plant (Fig. 6.1A). To date, draft reference genome sequences have been completed and/or published for nearly all the major types of agricultural vectors including whiteflies (Chen *et al.*, 2016), planthoppers (Zhu *et al.*, 2017), aphids (Consortium, 2010; Wenger *et al.*, 2017), and psyllids (Saha *et al.*, 2017). Whole genome studies have shed light on the gene families in these insects that are critical for plant adaptation, insecticide resistance, and virus transmission (Consortium, 2010; Chen *et al.*, 2016; Kaur *et al.*, 2016; Wenger *et al.*, 2017; Zhu *et al.*, 2017). Functional annotation of genes coded by

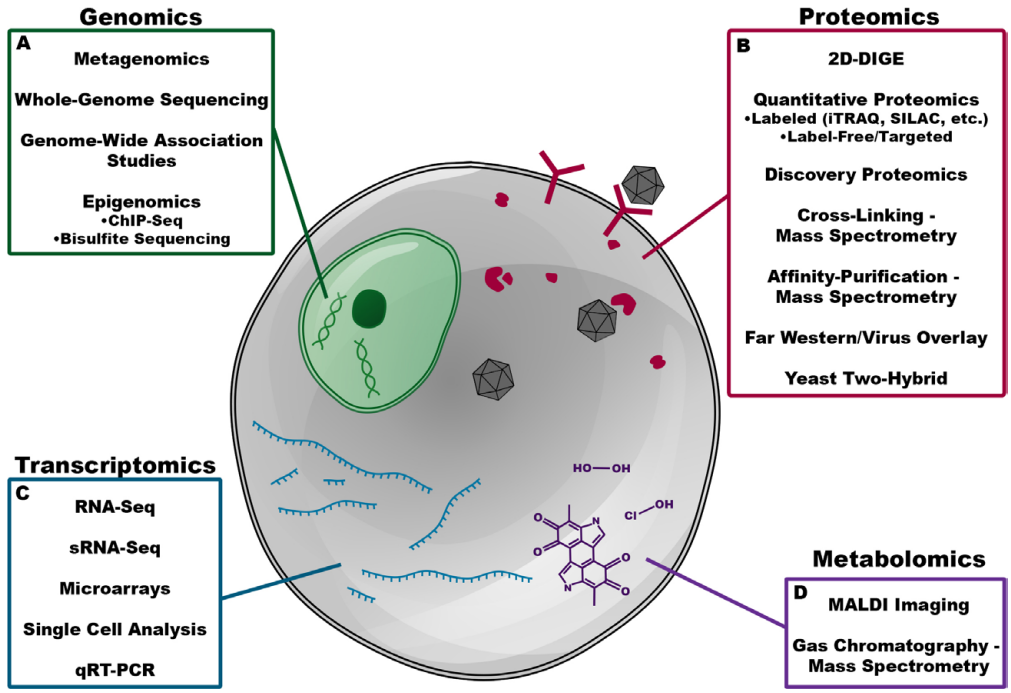

Figure 6.1 'Omics approaches to studying vector biology, including (A) genomics; (B) proteomics; (C) transcriptomics; and (D) metabolomics. 2D-DIGE, 2-dimensional difference gel electrophoresis; ChIP-seq, chromatin immunoprecipitation sequencing; iTRAQ, isobaric tags for relative and absolute quantification; MALDI, matrix-assisted laser desorption/ionization; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction; RNA-seq, RNA sequencing; SILAC, stable isotope labelling by amino acids in cell culture; sRNA-seq, small RNA sequencing.

vector genomes is still highly reliant on homology-based methods to other arthropods (Oppenheim *et al.*, 2015), especially the model species *Drosophila melanogaster* (Saha *et al.*, 2017), which means many genes in non-model vectors lack a validated functional assignment. Structural annotations also need to be improved for these data to be more useful to vector biologists.

Genome sequence information is used as the foundation for the analysis of other types of 'omics data: transcriptomics, proteomics, and metabolomics (Fig. 6.1B–D). Transcriptomics (Fig. 6.1C), the most widely used 'omics technique in biology, involves the detection and quantification of gene expression at the RNA level that occurs during certain developmental stages and/or in response to changes in physiological conditions. There are several RNA species that can be measured including mRNA, small RNAs, long non-coding RNAs, and viral RNA, with each requiring slight variations in sample preparation and downstream bioinformatics analysis (Kukurba and Montgomery, 2015). These types of studies provide evidence for the putative involvement of those genes and/or their gene products with the insect's developmental stage (Wang *et al.*, 2010) or other conditions being studied. These can include changes in gene expression that occur during virus acquisition compared with non-viruliferous insects (Brault *et al.*, 2010), comparisons of the saliva of different aphid vector species (Thorpe *et al.*, 2016), the response of insects being reared on different host plants (Christodoulides *et al.*, 2017; Mathers *et al.*, 2017), or the molecular mechanisms associated with insecticide resistance (Yang *et al.*, 2013). Expressed sequence tags (EST) and microarrays were extensively used in the past for gene expression studies in vector species (Ramsey *et al.*, 2007; Brault *et al.*, 2010; Götz *et al.*, 2012). Now, next-generation sequencing techniques are emmployed. Proteome studies (Fig. 6.1B) often measure proteins using mass spectrometry (MS). These studies investigate gene expression at the translational level and identify post-translational modifications, which lead to

differences in the levels of proteins being produced under a physiological condition or their localization within cells. Discovery-based proteomics techniques allow the researcher to identify the proteins present in a sample by matching peptide sequences to databases of known protein sequences (derived from the genome or transcriptome data) (Cilia *et al.*, 2011b) and to quantify their levels relative to other samples (Pinheiro *et al.*, 2014). Some techniques in proteomics allow one to measure the levels of a specific target protein (targeted experiments) (Cilia *et al.*, 2012a) or identify and quantify protein–protein interactions (co-immunoprecipitation-MS and crosslinking-MS technology) (Chavez *et al.*, 2012; DeBlasio *et al.*, 2015a). Metabolomics (Fig. 6.1D) is the study of the metabolome of an organism, that is, all the metabolites produced by an organism, tissue, or cell under a physiological condition. Metabolic outputs are downstream to gene transcription and protein expression. Methods may be targeted to measure individual metabolites with known standards, or non-targeted, enabling the discovery of new metabolites and metabolic signatures (Maag *et al.*, 2015). Combining 'omics techniques can provide a more complete picture of what is occurring on the molecular level. For instance, proteomics coupled to transcriptomics can give a more nuanced picture of how proteins are regulated on the post-transcriptional and post-translational level, independent of gene expression (Kruse *et al.*, 2017). Metabolomics can also be combined with transcriptomics and/or proteomics to understand how the regulation of enzymes gives rise to the differential expression of metabolites (Kersten *et al.*, 2013).

Plant virus transmission by insects involves a series of carefully orchestrated, temporally-regulated protein interactions among virus, vector, and the plant host, all of which can be captured and measured quantitatively using these various 'omics approaches. The first step in virus transmission is acquisition, or the retention of the virus in the vector. Acquisition may be as simple as virus transiently binding to insect mouthparts, or as complicated as virus transcytosis across the insect gut. Protein interaction studies have been used to identify viral, vector and host components involved (detailed in sections below). Next, there may be a latent period during which the virus must circulate through the vector, which, for the propagative viruses, includes virus replication. RNA sequencing and proteomics have been used to measure the various effects viruses and virus-infected plants have on their insect vectors. Finally, the virus must be inoculated into a new host, completing the transmission process. Protein interaction studies (such as in Fig. 6.1B), ultrastructural studies, classical molecular biological and entomological approaches (for example, electrical penetration graph) have been used to study the latter step. Modes of transmission are often defined by the length of these various steps of the process, as well as the vector tissues where the virus is retained following acquisition.

The terminology in the vector biology literature can be as confusing and full of jargon as the 'omics literature, so we attempt to clarify a few key points here when discussing insect vectors. In this chapter, we will follow the designations provided by Gray and Banerjee (1999): that the modes of transmission should be classified as 'circulative' or 'non-circulative', depending on whether the virus passed through insect vector cell membranes and was retained internally within the vector, which would constitute circulative transmission. Not all vector species transmit all viruses and not all individuals within a species are capable of transmitting a particular virus species. This natural variation in vectoring ability is found in many vector species and is genetically encoded (reviewed in Gray *et al.*, 2014). In these vector species, vector and non-vector are common terms to describe individual insects (or vector species), which are either capable or not capable, respectively, of efficient virus transmission. Finally, insects which have acquired circulative viruses are referred to as viruliferous. Insects which have acquired circulative, propagative viruses (which replicate in the insect vector as well as the plant host) may also be referred to as infected.

New knowledge in vector biology 'omics includes the identification of direct protein interactions facilitating virus transmission as well as indirect effects, such as host and vector manipulation (detailed in sections below). Whereas the viral components to transmission have largely been characterized by more traditional molecular biology techniques and microscopy, rapid advances in 'omics technologies have fuelled discovery on the vector side of vector–pathogen interactions (Heck, 2018) and have led to the development of novel strategies that block specific molecular events involved in the steps of virus

transmission (Heck and Brault, 2018). Advances in RNA interference (RNAi) in non-model insects have also opened the door to functional validation of candidate genes and proteins involved in virus transmission by hemipteran insect vectors (Pitino *et al.*, 2011; Mulot *et al.*, 2018).

Virus–vector protein interactions involved in non-circulative transmission

Viruses transmitted in a non-circulative mode are characterized by rapid acquisition and retention in the vector mouthparts and foregut. There is no latency period between acquisition and inoculation, and limited virus persistence in the vector. To date, aphids are the only known vectors of viruses transmitted in this mode. Non-circulative virus transmission is intimately linked to aphid probing behaviour (Drucker and Then, 2015). In seeking a new plant host, aphids penetrate the cell epidermis with their stylets in a series of shallow probes that puncture cell membranes, allowing the aphid to 'taste' cellular components and to come into direct contact with virions. Virions bind the aphid mouthparts or foregut and can be acquired in a matter of seconds to minutes. The virions can later be dislodged from aphid mouthparts during the same probing process on a susceptible plant host. Therefore, aphids need not colonize a plant host to transmit virus in this mode. In fact, many non-circulative viruses are transmitted by non-colonizing aphids, and vector and virus host range do not entirely overlap (Berlandier *et al.*, 1997). While non-circulative viruses can be transmitted by many different aphid species, there is still some degree of specificity in virion binding to aphid mouthparts, which will be discussed in the subsections below.

Viruses using the capsid strategy

Transmission of non-circulative viruses can be broadly separated into two categories, depending on the viral proteins required: the capsid strategy or the helper component strategy. Early work on non-circulative viruses by Pirone and Megahed (1966) involved feeding gradient-purified virions to insects via a membrane sachet and testing if transmission occurs. For some viruses, this membrane feeding was successful, indicating that virion proteins alone were sufficient for transmission. Virions were thought to directly bind aphid mouthparts, in what has been termed the capsid strategy. *Cucumber mosaic virus* (CMV) is one of the best characterized among the non-circulative viruses to use the capsid strategy. CMV has a tripartite genome, and when reassortment of viral RNA segments occurs in mixed infections, the transmission phenotype always corresponds to the virus contributing RNA 3, which encodes the capsid coat protein (CP) (Mossop and Francki, 1977). Mutational analysis of natural and engineered variants of the CMV CP has identified many residues that are required for transmission by the aphid vectors, *Aphis gossypii* and *Myzus persicae* (Perry *et al.*, 1994, 1998; Liu *et al.*, 2002). Some of these mutations abolish aphid transmission by affecting virion stability, indicating that properly assembled virus particles are required for transmission (Ng *et al.*, 2000, 2005). The highly-conserved βH-βI loop of the capsid surface, which is comprised almost entirely of acidic residues, has also been found to be involved in transmission, as mutations that lead to substitutions of neutral or basic residues in this region abolish transmission (Liu *et al.*, 2002). Additionally, these virus mutants could regain transmission ability if compensatory residue substitutions occurred elsewhere in the capsid, which may have preserved virion stability or charge distribution (Liu *et al.*, 2002).

Progress has been slow in identifying insect proteins involved in the transmission of non-circulative viruses, largely due to the difficulty in identifying and extracting cuticle proteins as well as the small size of insect mouthparts. Only recently have vector proteins involved in transmission of non-circulative viruses been discovered. Liang and Gao (2017) performed yeast two-hybrid assays to detect the binding of *M. persicae* cuticle proteins with the capsid of CMV. The cuticle protein *M. persicae* cuticle protein 4 (MPCP4) was found to directly interact with the CMV CP (Fig. 6.2G). Knockdown of MPCP4 by double stranded RNA (dsRNA) feeding led to less acquisition of CMV CP in the aphids, as detected by quantitative polymerase chain reaction (qPCR), indicating that MPCP4 may be an aphid receptor of CMV (Liang and Gao, 2017). This same *M. persicae* protein was recently found to be involved in the transmission of *Cauliflower mosaic virus* (CaMV), a helper

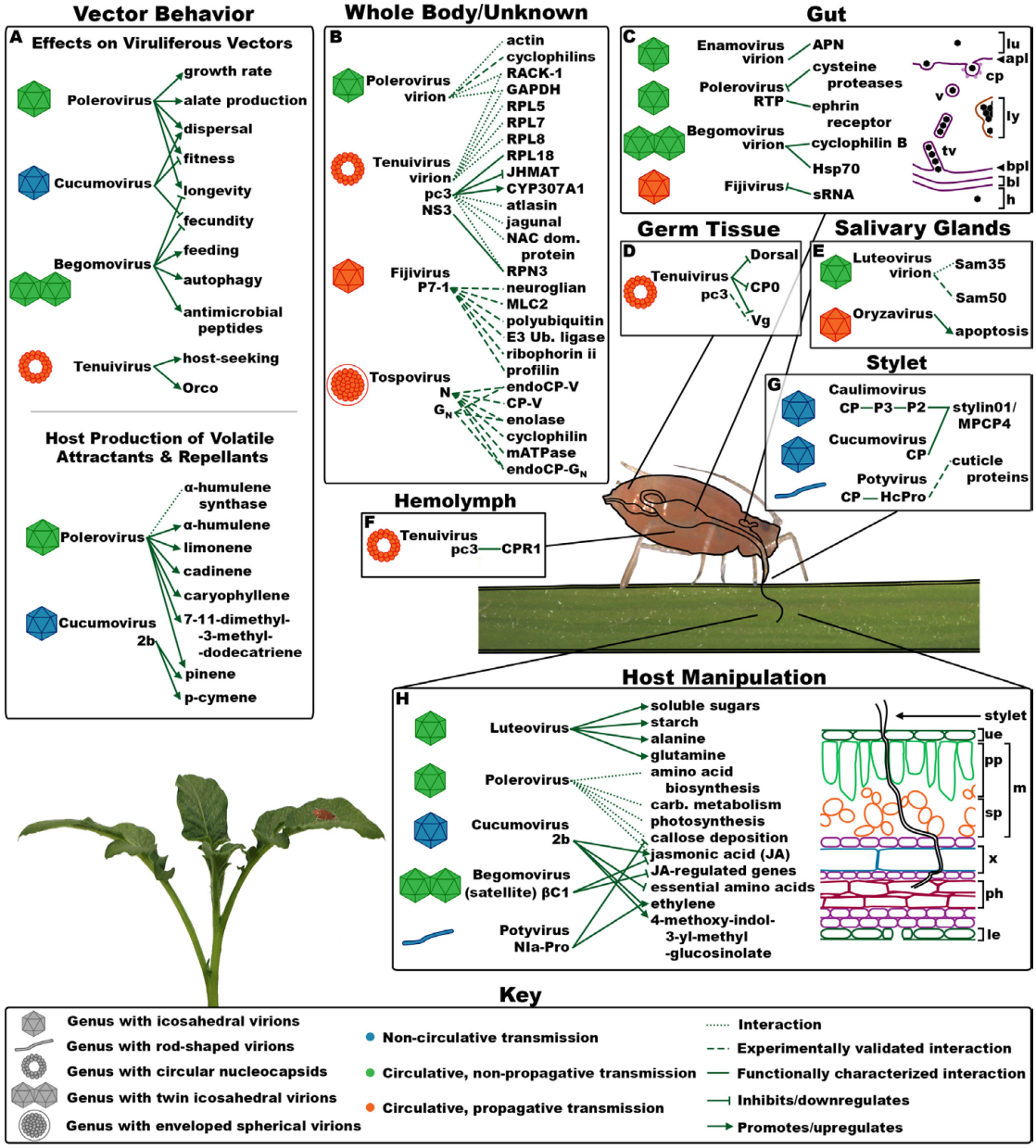

Figure 6.2 Overview of interactions between virus, vector and host involved in the transmission of plant viruses, as determined using 'omics technologies. (A) Interactions affecting vector behaviour, including the production of volatile attractants. (B) Interactions between virus and vector proteins with unknown or undetermined localization. (C) Interactions between virus and vector proteins and processes occurring in the insect gut. Diagram at right shows a model for virion movement across the gut. apl, apical plasmalemma; bl, basal lamina; bpl, basal plasmalemma; cp, coated pit; h, hemolymph; lu, lumen; ly, lysosome; tv, tubular vesicle; v, vesicle. (D) Interactions between virus and vector proteins occurring in the germ tissue. (E) Interactions between virus and vector proteins and processes occurring in the salivary glands. (F) Interactions between virus and vector proteins occurring in the hemolymph. (G) Interactions between viruses and vector proteins occurring in the stylet. (H) Interactions between virus and plant host factors affecting vector transmission. Diagram at right shows a cross-section of a hemipteran feeding on a leaf; le, lower epidermis; m, mesophyll; ph, phloem; pp, palisade parenchyma; sp, spongy parenchyma; ue, upper epidermis; x, xylem. Key for colour and symbol codes; left column: symbol for virion structure; centre column: transmission mode indicated by virion symbol colour; right column: edge types denoting different types of virus–host or virus–vector interactions.

component strategy virus (see below) (Webster *et al.*, 2018).

Viruses using the helper component strategy

Not all viruses can be transmitted as purified virions alone. Work by Kassanis and Govier (1971) identified a non-transmissible form of *Potato virus Y* (PVY) that could be conditionally transmitted if aphids were first given an acquisition period on wild-type PVY, but not if acquired in the reverse order. Therefore, they concluded that aphids captured a 'helper component' from the wild-type strain. This viral protein was later identified and named HC-Pro (for helper component-protease). Govier and Kassanis (1974) could reconstitute aphid transmission by purifying HC-Pro from plants and mixing it with purified virions in membrane feeding assays. Non-circulative viruses that require one or more non-structural viral proteins for transmission are said to use the helper strategy.

Since the discovery of helper components in the potyvirus PVY, there have been advances in understanding the mechanism of transmission for the *Potyviridae.* Potyviruses have filamentous rod-shaped capsids composed of a single CP (Zamora *et al.*, 2017). During transmission, the CP is bound by HC-Pro, which is a multifunctional viral protein with protease activity (Ivanov *et al.*, 2016). HC-Pro forms a 'molecular bridge' between the capsid and the aphid stylet (Ammar *et al.*, 1994). A surface-exposed domain in the N-terminus of the CP and the amino acid sequence motif DAG have been found to be highly conserved across potyviruses and necessary for aphid transmission (Allison *et al.*, 1985; Harrison and Robinson, 1988; Shukla *et al.*, 1988; Atreya *et al.*, 1991, 1995). The DAG motif is thought to bind the PTK amino acid motif near the C-terminus of HC-Pro (Peng *et al.*, 1998). KITC, near the N-terminus of HC-Pro, is another highly-conserved amino acid motif that has been tied to aphid transmission and is thought to bind the aphid mouthparts (Peng *et al.*, 1998).

CaMV, an icosahedral virus in the *Caulimoviridae* family, also binds insect mouthparts using the helper strategy. However, it requires not one, but two viral proteins in addition to the capsid: P2 and P3 (Woolston *et al.*, 1983). Using far western blot analysis, the P3 protein was found to directly bind virion (Leh *et al.*, 2001; Plisson *et al.*, 2005) and P2 (Leh *et al.*, 1999), while the P2 protein is thought to coordinate the interaction between virions. This viral protein forms aggregates termed 'transmission bodies' (Espinoza *et al.*, 1991; Drucker *et al.*, 2002; Khelifa *et al.*, 2007) that are responsive to wounding, such as during aphid probing (Bak *et al.*, 2013; Martinière *et al.*, 2013). When an infected leaf is wounded, the transmission bodies dissociate and P2 disperses onto microtubules throughout the cell (Martinière *et al.*, 2013). Virions of CaMV are also shown to change localization in response to aphid probing and colocalize with P2 along the microtubules, purportedly to increase the probability of transmission (Bak *et al.*, 2013).

To date, few vector proteins have been identified to be involved in the transmission of non-circulative viruses using the helper strategy. Dombrovsky *et al.* (2007) extracted proteins from *M. persicae* and tested binding of these proteins to the HC-Pro of the potyvirus *Zucchini yellow mosaic virus* using protein membrane overlay. The authors identified nine proteins binding HC-Pro, four of which were identified to be cuticle proteins via analysis by MS (Fig. 6.2G). Their binding to HC-Pro, but not to the virus capsid, supports the role of these proteins in transmission, as potyviruses use the helper strategy, and the virus capsid alone is not sufficient to directly bind the aphid stylet. Additionally, these proteins failed to bind a mutant form of HC-Pro where the lysine residue in the KLSC domain was changed to glutamic acid, a residue substitution that also abolishes aphid transmission (Dombrovsky *et al.*, 2007). For CaMV, the *M. persicae* protein stylin-01 (formerly known as MPCP4) was identified as the receptor for the virus in the aphid stylet by competitive binding between a stylin-01 antibody and the CaMV P2 protein (Webster *et al.*, 2018) (Fig. 6.2G). Additionally, knockdown of the *stylin-01* gene by feeding aphids on short interfering RNAs (siRNAs) specific to *stylin-01* led to diminished transmission of CaMV by *M. persicae*. Considering this same protein was previously implicated in the transmission of CMV (Liang and Gao, 2017), it seems the same aphid cuticle protein can serve as a receptor for two non-circulative viruses using different transmission strategies. Further identification of receptors for non-circulative viruses in aphid mouthparts as well as characterization of protein interaction topologies using breakthrough proteomics technologies involving cross-linking

coupled to MS, such as Protein Interaction Reporter (Chavez *et al.*, 2012; DeBlasio *et al.*, 2015a), may lead to the development of interdiction strategies to block non-circulative virus transmission.

Virus-vector protein interactions involved in circulative, non-propagative transmission

Circulative, non-propagative transmission is characterized by a longer virus acquisition time of hours to days, a latent period during which the virus circulates within the insect, and no replication of the virus within the insect vector tissues. Insects often remain viruliferous for their entire lifespan after acquisition. Viruses from three families are transmitted in this mode: the *Luteoviridae*, *Geminiviridae*, and *Nanoviridae*. Viruses in the *Luteoviridae* (referred to in this chapter as luteovirids) and nanoviruses are transmitted by aphids. Historically, viruses in the *Geminiviridae* have been known to be transmitted by whitefly, leafhopper or treehopper vectors, but recently a report described the cowpea aphid, *Aphis craccivora*, as a vector for *Alfalfa leaf curl virus* (Roumagnac *et al.*, 2015). Circulative viruses are often only acquired when vector insects initiate phloem feeding (Fig. 6.2H). Phloem limitation of circulative, non-propagative viruses is a hallmark feature shared among viruses in these families, which is thought to help promote transmission by their sap-sucking hemipteran vectors (Peter *et al.*, 2009; Gray *et al.*, 2014). Circulative transmission depends upon many species-specific protein interactions. Most circulative virus species are only transmitted by one or a few vector species with varying degrees of transmission efficiency (Rochow, 1969, 1970; Gray *et al.*, 2002; Gray, 2007;).

Much of what is known about this mode of transmission comes from the luteovirid pathosystems. Several ultrastructural studies using electron microscopy have detailed the transmission process of luteovirids on the cellular level (Gildow, 1987; Gildow, 1993; Garret *et al.*, 1993, 1996; Peiffer *et al.*, 1997) (Fig. 6.2C). After feeding on a source of virus, virions enter the gut lumen of the insect and are acquired through the gut epithelial cells. Different luteovirids are acquired through different parts of the gut; either the posterior midgut or hindgut (Garret *et al.*, 1993; Gildow, 1993). In contrast, viruses in the *Geminiviridae* are acquired across the filter chamber or midgut (Ghanim and Medina, 2007; Czosnek *et al.*, 2017), whereas nanoviruses have been observed to passage through the foregut and anterior midgut (Ghanim and Medina, 2007; Bressan and Watanabe, 2011). Virions travel through the endomembrane system of gut epithelial cells in the form of coated pits or tubular vesicles (Gildow, 1993; Garret *et al.*, 1993, 1996). Once the virions are trafficked through the cell, they are released at the basal plasmalemma via exocytosis and pass through the basal lamina into the hemocoel, where they diffuse through the open circulatory system of the vector (Garret *et al.*, 1993, 1996) until they reach the salivary glands. The route used by these viruses in the hemolymph from the gut to the salivary tissues is a largely unexplored and understudied research area. At the primary or accessory salivary glands, virions are again endocytosed and trafficked through vesicles until they reach the posterior cell membranes where they are released into the salivary canal. Luteovirids enter the accessory salivary gland (Peiffer *et al.*, 1997), whereas viruses in the *Geminiviridae* and nanoviruses pass into the primary salivary glands (Ghanim and Medina, 2007; Bressan and Watanabe, 2011). Finally, virions are injected into the next host with salivary secretions for feeding (Peiffer *et al.*, 1997), completing the process of transmission.

Protein interactions regulating acquisition and transmission of luteovirids

Only the structural proteins of the luteovirid capsid have been shown to be involved in directly facilitating the circulative movement of virions through their aphid vectors. Luteovirid icosahedral virions are comprised of two proteins, a CP that makes up the majority of the 180 subunits of the capsid, and the readthrough protein (RTP), which comprises a minor but unknown number of monomer units. The RTP is generated by sporadic ribosomal readthrough of an amber stop codon at the end of the CP open reading frame, leading to the generation of a protein extension known as the readthrough domain (RTD) that protrudes from the surface of the virion. Domains in both the luteovirid CP and N-terminal region of the RTD have been implicated in insect transmission (Chay *et al.*, 1996; Brault *et al.*, 2000, 2003, 2005; Reinbold *et al.*, 2001; Lee *et al.*, 2005; Kaplan *et al.*, 2007; Peter *et al.*, 2008;

Boissinot *et al.*, 2014). The roles for the luteovirid capsid proteins in insect transmission and plant infection have been extensively reviewed elsewhere (Gray *et al.*, 2014; Oppenheim *et al.*, 2015; Whitfield *et al.*, 2015; Heck and Brault, 2018). Protein interaction work using Protein Interaction Reporter (PIR) technology, a chemical crosslinking MS approach to measure protein interaction topologies, has shown that capsid proteins from different luteovirid species share common structural features in their virions (Chavez *et al.*, 2012; DeBlasio *et al.*, 2015a; Alexander *et al.*, 2017).

Virus overlay assays coupled to MS analysis have led to the identification of aphid proteins that can directly bind the virions of luteovirids (van den Heuvel *et al.*, 1994). These include five proteins from *M. persicae* homogenate found to bind virions of *Potato leafroll virus* (PLRV). One of these proteins, symbionin (a homologue of GroEL), is produced by the aphid obligate endosymbiont *Buchnera aphidicola*. However, recent work carried out by Bouvaine and colleagues show that symbionin is unlikely to come into contact with, nor would the predicted binding site be structurally accessible to, luteovirids in aphids (Bouvaine *et al.*, 2011), underscoring the importance of validating the role of protein interactions in virus transmission identified using *in vitro* approaches. For *Barley yellow dwarf virus-MAV* (BYDV-MAV), two aphid proteins found to bind virion, SaM35 and SaM50, were detected in the head of the aphid vector *Sitobion avenae* (Li *et al.*, 2001) (Fig. 6.2E). Yet, proteins isolated from the heads of the aphid *Rhopalosiphum maidis*, a non-vector species of this virus, were found not to bind MAV (Li *et al.*, 2001). SaM50 from *S. avenae* and *Schizaphis graminum* was also found to directly interact with BYDV-GDV, which is only transmitted by these two aphid species (Wang, 2003). Immunogold labelling of ultrathin sections of aphid heads showed that SaM50 localized to the plasma membrane of the accessory salivary gland of *S. graminum* but not in the non-vector species *Rhopalosiphum padi*. In addition, aphid feeding on artificial diets containing antibodies towards this protein reduced transmission of BYDV-GAV by both *S. avenae* and *S. graminum*. Using the same far western blot technique, Seddas *et al.* (2004) identified several proteins binding to *Beet western yellows virus*, including symbionin, Rack-1, GAPDH, and actin (Fig. 6.2B), although their function in aphid transmission has yet to be determined. Microarray analysis of gene expression in the gut of pea aphids after acquisition of *Pea enation mosaic virus 1* (PEMV1) identified several differentially expressed transcripts with limited fold change of up- or down-regulation, including genes involved in endocytosis, intracellular trafficking and signal transduction, all important processes in virus acquisition (Brault *et al.*, 2010). Only 20% of the genome was represented in this transcriptomic analysis due to the limited availability of aphid genome sequences at that time and the low enrichment of gut-specific transcripts in the microarray (Brault *et al.*, 2010). Further transcriptomic analysis is needed to determine if this low level of differential expression is due to the limitations of the study or if aphid gut physiology is not greatly perturbed by luteovirid acquisition.

A powerful way to identify proteins involved in transmission is proteomic phenotyping by comparing the proteomes of vector and non-vector aphids within a species. Papura *et al.* (2002) first performed this method using 2-D gel electrophoresis on an F1 population from a selfed *S. avenae* clone differing in BYDV-PAV transmission efficiency. They identified two differentially expressed protein spots with dissimilar isoelectric points but identical molecular weights between vector and non-vector aphids, indicating that these differential spots were isoforms of the same protein most likely derived from one biallelic locus (Papura *et al.*, 2002). Advances in 2-D proteomics methods in the early 2000s, namely in the development of Difference In Gel Electrophoresis (DIGE), which relies on the use of an internal standard included in every gel for relative quantification of all samples in the study, enabled highly precise, quantitative comparison of proteomes from across a large number of samples. Comparative proteomic profiling by 2-D DIGE of an F2 population of *S. graminum* differing in transmission ability of the luteovirid *Cereal yellow dwarf virus-RPV* (CYDV-RPV) also identified differentially expressed protein isoforms from biallelic loci that correlated with transmission phenotype and could even pinpoint which proteins were involved in passage across the gut as compared to the accessory salivary gland (Yang *et al.*, 2008; Cilia *et al.*, 2011a). One such protein, cyclophilin B, was present in two isoforms, S28 and S29, differing in a single amino acid change (Tamborindeguy *et*

al., 2013). Only the S29 isoform was found to be present in genotypes and field-collected aphids that were efficient transmitters of CYDV-RPV (Tamborindeguy *et al.*, 2013). Additionally, both S28 and S29 were found to bind CYDV-RPV *in vitro* and in co-immunoprecipitations from aphid tissue (Fig. 6.2B), but could not bind PLRV *in vitro*, for which *S. graminum* is a non-vector (Tamborindeguy *et al.*, 2013). Co-immunoprecipitations from aphid tissue coupled to MS also detected another cyclophilin protein binding CYDV-RPV, cyclophilin A, indicating a larger role for cyclophilins in the transmission of this virus.

Recent work in luteovirid systems has discovered the first putative receptors for circulative virus transmission within the gut of aphids. In a hallmark paper by Linz *et al.* (2015), aminopeptidase-N (APN) from pea aphid, *Acyrthosiphon pisum*, was identified to bind PEMV1 using a 2-D far western blot method coupled to MS (Fig. 6.2C). This interaction was confirmed via co-immunoprecipitation, immunofluorescence binding assays, and surface plasmon resonance (Linz *et al.*, 2015). The role of APN as a receptor was confirmed *in vitro* by showing the internalization of green fluorescent protein (GFP)-labelled PEMV1 CP by Sf9 insect cells expressing APN from pea aphid (Linz *et al.*, 2015). Additionally, Linz *et al.* (2015) showed that PEMV1 competes with a peptide that also binds APN, GBP3.1, which was previously shown to inhibit the uptake of this virus by aphids (Liu *et al.*, 2010). Recently, a second luteovirid receptor, the ephrin receptor from *M. persicae*, was found to unambiguously interact, via yeast two-hybrid screening, with the RTP of *Turnip yellows virus* (TuYV) (Mulot *et al.*, 2018) (Fig. 6.2C). Knockdown of ephrin in aphids by dsRNA feeding reduced transmission of TuYV by 43–77%, providing *in vivo* evidence of its role in transmission (Mulot *et al.*, 2018). Additionally, the authors found evidence of this receptor binding to the *Cucurbit aphid borne yellows virus* (CABYV) CP and RTP via yeast two-hybrid screening. The knockdown of the ephrin receptor in *M. persicae* reduced acquisition of CABYV as well as *Beet mild yellowing virus*, two other closely related luteovirids primarily transmitted by *M. persicae*, suggesting its broader role as a general receptor for luteovirids in this widespread vector (Mulot *et al.*, 2018).

Protein interactions regulating acquisition and transmission of begomoviruses

Begomoviruses, in the *Geminiviridae* family, are circulatively transmitted by whitefly vectors and may have mono- or bipartite genomes. While historically referred to as non-propagative, there is some possible evidence for virus replication in vector tissues (Pakkianathan *et al.*, 2015). The CP is thought to be the sole viral determinant of plant-to-plant transmission in begomoviruses, supported by changes in transmission specificity when CP sequences are swapped between geminiviruses. For instance, replacing the CP of *African cassava mosaic virus* (ACMV) with the CP of the leafhopper-transmissible *Beet curly top virus* resulted in a leafhopper-transmissible ACMV (Briddon *et al.*, 1990). Furthermore, exchanging the CP of the non-transmissible *Abutilon mosaic virus* (AbMV) with that of the transmissible *Sida golden mosaic virus* rendered AbMV transmissible (Höfer *et al.*, 1997). The CP of geminiviruses have been shown to bind the midgut of their respective insect vectors, the site of virus acquisition (Wang, Y. *et al.*, 2014). Feeding on antibodies raised against the CP alone or those specific for assembled virion inhibited transmission of *Wheat dwarf virus* by its leafhopper vector (Wang, Y. *et al.*, 2014). Detailed mutational analysis of begomovirus CPs has identified many residues that are important for transmission, especially the amino acids between residues 129 and 152 (Azzam *et al.*, 1994; Noris *et al.*, 1998; Liu *et al.*, 1999; Höhnle *et al.*, 2001; Soto *et al.*, 2005; Caciagli *et al.*, 2009). As for other plant viruses, proper virion assembly and stability is important for transmission: the CP of *Tomato yellow leaf curl Sardinia virus* with an asparagine to aspartate mutation at position 130 was unable to assemble and was not acquired by its whitefly vector, *Bemisia tabaci* (Caciagli *et al.*, 2009).

Two vector proteins have been implicated in regulating transmission of begomoviruses by *B. tabaci*. Microarray, qPCR, and western blot analysis showed an up-regulation of heat shock protein 70 (Hsp70) in response to *Tomato yellow leaf curl virus* (TYLCV) and *Squash leaf curl virus* (Götz *et al.*, 2012). Immunocapture PCR, virus overlay assays, and co-immunoprecipitation showed that Hsp70 interacts with TYLCV (Fig. 6.2C). Immunostaining

showed co-localization of Hsp70 with TYLCV and *Watermelon chlorotic stunt virus* in whitefly midgut epithelial cells (Götz *et al.*, 2012). Finally, the role of Hsp70 in virus transmission was confirmed functionally by feeding whiteflies on antibody raised against Hsp70 prior to virus acquisition. This antibody feeding assay resulted in increased transmission of TYLCV, indicating that Hsp70 may be a negative regulator of transmission and serves to protect whiteflies against begomoviruses (Götz *et al.*, 2012). Cyclophilin B was identified as a positive regulator of TYLCV transmission by *B. tabaci* (Kanakala and Ghanim, 2016) (Fig. 6.2C). Similar feeding studies using a cyclophilin B antibody resulted in a decrease in TYLCV transmission. Delivery of cyclosporin A, a cyclophilin B inhibitor from the fungus *Tolypocladium inflatum*, to whiteflies via artificial diet decreased co-localization of cyclophilin B and TYLCV in whitefly midguts (Kanakala and Ghanim, 2016).

Virus–vector protein interactions regulating nanovirus acquisition and transmission

Viruses in the family *Nanoviridae* are multipartite, with genomes consisting of six to eight single-stranded DNA segments, each individually encapsidated into icosahedral particles comprised of a single coat protein. Progress in genetically dissecting these viruses has been slowed by the difficulty in generating infectious clones of the many genome segments. Unlike the other viruses transmitted in the circulative, non-propagative mode, early studies showing the inability of purified nanovirus particles to be transmitted by aphids indicated that a helper component could be required (Franz *et al.*, 1999). This component was recently identified in *Faba bean necrotic stunt virus* (FBNSV) to be the nuclear shuttle protein (NSP) encoded by DNA-N (Grigoras *et al.*, 2018). The authors found that FBNSV was no longer aphid-transmissible when the infectious clone containing the DNA-N viral segment, which encodes the NSP, was excluded from plant inoculation (Grigoras *et al.*, 2018). How the NSP protein facilitates transmission is unknown. However, the function of this viral protein is conserved, as the NSP of FBNSV could complement that of the closely related *Faba bean necrotic yellows virus* and the distantly related *Pea necrotic yellow dwarf virus* (Grigoras *et al.*, 2018). Moreover, the mechanism may be distinct from the role of helper components in non-circulative viruses, as NSP was found to not directly bind the CP in yeast two-hybrid and bimolecular fluorescence complementation assays (Krenz *et al.*, 2017). To date, aphid proteins involved in nanovirus transmission have not been reported. However, a recent study has shown that different genome segments of nanoviruses exhibit unequal relative frequencies in their aphid vectors compared with in plants (Gallet *et al.*, 2018). Further understanding of virus and vector proteins that regulate the transmission of nanoviruses is an exciting frontier, considering the unique challenges presented by their extreme multipartite virus lifestyle.

Virus–vector interactions regulating circulative, propagative transmission

Plant viruses transmitted in the circulative, propagative mode are characterized by acquisition times of hours to days, a latent period of days to weeks during which the virus replicates and disseminates within the vector, and long-term persistence in the vectors, which remain viruliferous for their entire lives. The route taken by circulative, propagative viruses through their vector often mirrors that taken by circulative, non-propagative viruses: virions pass through the gut epithelial cells into the hemolymph, then enter the salivary glands, with the key distinction that propagative viruses replicate in nearly every tissue encountered, notably the gut and salivary glands. Propagative viruses also replicate in other vector tissues not directly involved in the circulative pathway for non-propagative viruses, such as muscle cells adjacent to the gut, from which virions can pass into the hemolymph. These viruses can also directly enter the salivary glands in vectors and developmental stages where these tissues are in contact, such as in thrips nymphs (Kritzman *et al.*, 2002; Moritz *et al.*, 2004). Viruses transmitted in a propagative mode often infect the ovarioles and can be vertically transmitted between generations of the vector (Huo *et al.*, 2014). Some viruses transmitted in this mode can produce tubules that facilitate escape from the midgut (Liu *et al.*, 2011; Chen *et al.*, 2012; Mar *et al.*, 2014; Wang, H. *et al.*, 2014).

Virus–vector protein interactions regulating rhabdovirus acquisition and transmission

Plant-infecting viruses in the *Rhabdoviridae* family (genera *Nucleorhabdovirus* and *Cytorhabdovirus*) are transmitted in a circulative, propagative mode by planthopper, leafhopper, and aphid species. Considering this mode of transmission, rhabdoviruses replicate in both their plant hosts and insect vectors (reviewed in Jackson *et al.*, 2005). Almost all viral proteins in this family are thought to act similarly in the two hosts, except for the movement proteins, which are used exclusively in the plant to transverse plasmodesmata (Huang *et al.*, 2005). Rhabdovirus capsids are enveloped in a membrane derived from the insect or plant host with viral-encoded tripartite glycoprotein (G) spikes protruding from these membranes. For rhabdoviruses that infect vertebrates, such as *Vesicular stomatitis virus*, the G protein is involved in facilitating the adhesion and entry into cells (Schlegel and Wade, 1983). A seminal paper by Gaedigk *et al.* (1986) showed that antibodies raised against the G protein of *Potato yellow dwarf virus* (PYDV) neutralized the infectivity of PYDV by preventing entry of the virus into vector cells. Therefore, the G protein of plant-infecting rhabdoviruses likely functions similarly to the G protein in vertebrate-infecting viruses of the same family.

A few transcriptomic studies have begun to investigate vector pathways involved in the transmission of plant rhabdoviruses. Comparing gene expression between *Maize mosaic virus* (MMV)-infected *Peregrinus maidis* and uninfected planthoppers revealed down-regulation of four genes involved in innate immune responses: the autophagocytosis associated protein ATG3, phosphoinositide 3-kinase (PI3K), c-JUN NH2-terminal kinase (Jnk), and tripeptidyl-peptidase II (TPPii) (Whitfield *et al.*, 2011). In a comparative transcriptomic study of *Graminella nigrifrons*, the vector of *Maize fine streak virus* (MFSV), insects were separated according to whether they were vectors (based on the results of a virus transmission assay to plants), and infected or uninfected. Vectors could be distinguished by the down-regulation of three peptidoglycan recognition proteins (PGRPs), which are involved in innate immune responses to bacteria and fungi (Chen *et al.*, 2012). A later study also implicated innate immunity genes in MFSV transmission by *G. nigrifrons* (Cassone *et al.*, 2014). After just four hours, the authors observed an up-regulation of many immune response genes, including genes involved in the Toll pathway, peroxidases, a superoxide dismutase, and PGRPs, in addition to genes related to cytoskeleton organization, hemopoiesis, glycosylation, and phagocytosis. However, this immune response was transient, as, by the seven-day time point, transcript levels had returned to the same levels as in the healthy controls (Cassone *et al.*, 2014). Emerging transcriptome data for vector species reveal homologues to proteins known to interact with vertebrate-infecting rhabdoviruses. For instance, the *P. maidis* genome contains a homologue of the nicotinic acetylcholine receptor, which is thought to bind and facilitate entry of *Rabies virus* (Whitfield *et al.*, 2011). No rhabdovirus receptor has been identified in insects.

Virus-vector protein interactions regulating tospovirus acquisition and transmission

Tospoviruses (in the newly-designated family *Tospoviridae*) are enveloped, negative-sense RNA viruses with a tripartite genome that are transmitted by thrips in a circulative, propagative mode. The tospovirus genome is protected by the nucleoprotein (N), which is surrounded by a host-derived membrane embedded with two tospovirus-encoded glycoproteins, G_N and G_C, that are involved in transmission. In reassortments, virus transmission determinants always map to RNA M, which encodes the glycoproteins (Sin *et al.*, 2005). Additionally, mutations in the G_N/G_C open reading frame abolish thrips transmission without altering virus assembly (Zheng *et al.*, 2011). G_N plays a role in virus attachment to the thrips midgut as evidenced by immunolocalization and virus overlay assays (Ullman *et al.*, 1995; Bandla *et al.*, 1998; Kikkert *et al.*, 1998). G_C is hypothesized to be a viral fusion protein based on structure (Garry and Garry, 2004) and the fact that it undergoes pH-induced conformational changes typical of type II fusion proteins (Whitfield *et al.*, 2005). Functional analysis confirmed that G_C mediates fusion of host and virus membranes (Plassmeyer *et al.*, 2005, 2007).

There have been several transcriptomic and one proteomic analysis characterizing the effects that

tospoviruses have on their thrips vectors. Badillo-Vargas *et al.* (2012) used transcriptome data to help identify proteins resolved using 2-D gel electrophoresis in uninfected and *Tomato spotted wilt virus* (TSWV)-infected *Frankliniella occidentalis.* The transcriptome was particularly useful for protein identification in this study as thrips genes have remarkably low similarity to genes found in other sequenced insect genomes. The authors identified 37 differentially expressed proteins between virus treatments, 62% of which were down-regulated. Many of these proteins were identified to be components of innate immune responses. Another *F. occidentalis* transcriptome analysis looking at the thrips response to TSWV revealed up-regulation of genes involved in defense, signal transduction, and endocytosis (Zhang *et al.*, 2013). Two later 'omics studies identified genes differentially expressed among the various developmental stages of *F. occidentalis* and *Frankliniella fusca* in response to TSWV. Attaining resolution at the level of developmental stage is crucial for tospoviruses, as they are only acquired during the larval stages (Ullman *et al.*, 1992; Nagata *et al.*, 1999). The authors found that in *F. occidentalis*, there was a global down-regulation of TSWV-responsive transcripts in the first instar larvae, including those involved in proteolysis and detoxification, which were up-regulated in the prepupal stage (Schneweis *et al.*, 2017). Cuticle proteins were one of the largest groups of TSWV-responsive transcripts, largely down-regulated and unique to larval and prepupal stages (Schneweis *et al.*, 2017). In *F. fusca*, transcripts of proteins involved in immune response, intracellular transport, and virus replication were found to be up-regulated (Shrestha *et al.*, 2017). Collectively, these studies have identified vector proteins potentially involved in tospovirus transmission and may serve to launch further investigation.

In an exciting preprint by Badillo-Vargas *et al.* (2018), the first ever tospovirus-binding thrips proteins were identified. Using 2-D gel overlay assays coupled to MS, the authors identified six thrips proteins from the first instar larvae that bind TSWV: the endocuticle structural glycoprotein endoCP-V, the cuticular protein CP-V, cyclophilin, enolase, mitochondrial ATP synthase α (mATPase), and the endocuticle structural glycoprotein endoCP-G_N (Fig. 6.2B). They confirmed binding of all six proteins to the TWSV N protein using yeast two-hybrid assays, and binding of endoCP-G_N to the TWSV glycoprotein G_N. These binding interactions were also validated via biomolecular fluorescence complementation. Furthermore, via immunolocalization, the authors confirmed the expression of these proteins in both the midgut and salivary glands of larval thrips, key tissues involved in tospovirus transmission. Co-localization of virus with cyclophilin and endoCP-G_N in the midgut, the site of tospovirus acquisition, was also observed. Considering the consistent and direct interaction between endoCP-G_N and TSWV G_N, the authors hypothesize that endoCP-G_N may serve some receptor-like role in the acquisition of tospoviruses, and future work should focus on pinpointing and verifying its function *in vivo*.

Virus–vector protein interactions regulating tenuivirus acquisition and transmission

Viruses in the genus *Tenuivirus* are transmitted by planthopper or leafhopper vectors in a circulative, propagative mode. The tenuivirus nucleocapsid protein pc3 has been found to be at the interface of interactions with the vector (described below). The non-structural viral protein NS4 forms inclusion bodies in insect tissues and directly interacts with pc3 and virus ribonucleoprotein complexes (Wu *et al.*, 2014a). RNAi targeting of NS4 shows that it is important for movement in the vector, but has no effect on replication (Wu *et al.*, 2014a).

Most of the research on tenuiviruses has focused on the type species, *Rice stripe virus* (RSV) transmitted by *Laodelphax striatellus*, the small brown planthopper. Several vector proteins have been found to interact with RSV. Using a dot immunobinding assay, *L. striatellus* proteins RACK1, GAPDH3, RPL5, RPL7, and RPL8 were found to interact with virus (Li *et al.*, 2011) (Fig. 6.2B). A Gal4 yeast two-hybrid screen was also used on the RSV system to look for interacting proteins localized to the nucleus, where they found binding between the RSV nucleocapsid protein pc3 and RPL18 (Li *et al.*, 2018) (Fig. 6.2B). Knockdown of RPL18 inhibited RSV translation and replication in the vector, indicating this vector protein is crucial for virus replication (Li *et al.*, 2018).

Recently, a split-ubiquitin yeast two-hybrid

screen between the RSV pc3 nucleocapsid protein and a library of small brown planthopper proteins identified atlasin, jagunal, NAC domain protein, a novel cuticular protein (CPR1), and vitellogenin (Vg) as interacting with pc3 (Liu *et al.*, 2015) (Fig. 6.2B). CPR1 was also found to co-localize with and bind virus *in vivo*. Knockdown of CPR1 using RNAi led to decreased RSV accumulation in the hemolymph and salivary gland as well as overall transmission (Liu *et al.*, 2015). The authors proposed that CPR1 binds to RSV and stabilizes it in the hemolymph (Liu *et al.*, 2015) (Fig. 6.2F). Vg was also identified as being down-regulated in RSV-viruliferous planthoppers in a separate transcriptomics study (Zhang *et al.*, 2010). On the protein level, Vg was found to co-localize with RSV *in vivo* (Huo *et al.*, 2014). RSV binding to Vg is thought to play a crucial role in vertical transmission by allowing RSV to enter nurse cells via endocytosis and ovarioles through the nutritive cords (Huo *et al.*, 2014) (Fig. 6.2D). Vg proteins may play a broader role in transmission of propagative plant pathogens through the manipulation of vector fecundity, as it has been found to be up-regulated in the hemolymph of *Diaphorina citri* (the Asian citrus psyllid) infected with '*Candidatus* Liberibacter asiaticus', (CLas) the Gram-negative, circulative, propagative bacterium associated with citrus greening disease (Kruse *et al.*, 2018). *D. citri* infection with CLas has a positive benefit on vector fitness for lab-reared colonies (Pelz-Stelinski and Killiny, 2016).

Small RNA sequencing of *L. striatellus* has shown that the vector produces viral-derived small interfering RNAs against RSV (Xu *et al.*, 2012a). Knockdown of the *Ago2* gene in planthoppers resulted in higher accumulation of RSV in the vector, indicating that RNAi-mediated antiviral immunity is active in the planthopper (Xu *et al.*, 2012a,b). RSV encodes a viral silencing suppressor protein, NS3, which is the most abundant viral transcript produced in the vector (Zhang *et al.*, 2010). Yeast two-hybrid experiments showed the RPN3 subunit of the 26S proteasome in the vector interacts with NS3 (Fig. 6.2B), and knockdown of NS3 results in increased RSV infection (Xu *et al.*, 2015). The authors hypothesize that NS3 hijacks the 26S proteasome by interacting with RPN3 to counter host defenses (Xu *et al.*, 2015).

Virus–vector protein interactions regulating reovirus acquisition and transmission

Three genera in the family *Reoviridae* infect plants (*Fijivirus*, *Phytoreovirus*, and *Oryzavirus*), and all are transmitted by planthoppers or leafhoppers in a circulative, propagative mode. Plant reoviruses are non-enveloped and icosahedral with an inner and outer capsid (Artimo *et al.*, 2012). The outer minor capsid protein of *Rice dwarf virus* (RDV), P2, contains features similar to the viral fusion proteins of other enveloped viruses, as indicated by syncytium formation when expressed in insect cells (Zhou *et al.*, 2007). Therefore, P2 is proposed to bind receptors in the midgut of the vector and facilitate virus entry by inducing fusion of the insect epithelial cell and viral membranes (Omura *et al.*, 1998). Interestingly, several plant reoviruses have been found to form tubule-like structures for movement in the vector. Tubules formed by RDV have been observed moving along microvilli in the midgut of its vector (Chen *et al.*, 2012) and crossing the basal lamina to the midgut visceral muscles, in the case of *Southern rice black-streaked dwarf virus* (SRBSDV) (Wang, H. *et al.*, 2014). The role of these tubules in virus movement was confirmed by knockdown of the tubule-forming protein P7–1 from SRBSDV, which inhibited tubule formation and virus spread in the vector (Liu *et al.*, 2011; Mar *et al.*, 2014).

SRBSDV is one of the most well-studied plant-infecting reoviruses. It is transmitted by the white backed planthopper, *Sogatella furcifera.* Transcriptomic analysis of gene expression between viruliferous and non-viruliferous insects showed that genes involved in primary metabolism, the ubiquitin-proteosome system, cytoskeletal organization, and immune pathways were responsive to SRBSDV (Xu *et al.*, 2012b). Another transcriptome study comparing insects varying in viral load identified the differential expression of transcripts involved in the induction of vector defense responses, with the RNA interference pathway being the most up-regulated (Wang *et al.*, 2016). *S. furcifera* proteins binding to the SRBSDV P7–1 tubule-forming protein were initially identified via yeast two-hybrid screening and 18 of the interactions were confirmed via chemiluminescent co-immunoprecipitation (Mar *et al.*, 2014). Organ-specific expression data gathered via quantitative

reverse transcriptase PCR (qRT-PCR) for six of these vector proteins (neuroglian, myosin light chain 2, polyubiquitin, E3 ubiquitin ligase, ribophorin ii, and profilin; Fig. 6.2B) were used to resolve the spatial organization of these virus–protein interaction networks (Mar *et al.*, 2014). Follow up studies should focus on determining the function of these proteins interacting with SRBSDV.

During transmission of circulative propagative viruses, infection in the vector advances from the midgut to midgut visceral muscles. Recent work by Lan *et al.* (2016a) shows that the RNA interference pathway is sufficient to prevent escape of SRBSDV from the midgut of the non-vector *L. striatellus* in cultured cells (Fig. 6.2C). However, knockdown of a key enzyme in this pathway, Dicer-2, allows virus accumulation in the midgut to reach the threshold necessary for dissemination to the visceral muscles (Lan *et al.*, 2016a). Interestingly, knockdown of Dicer-2 in whole insects even allowed the non-vector *L. striatellus* to transmit SRBSDV (Lan *et al.*, 2016a). A similar study on another reovirus system, *Rice gall dwarf virus* (RGDV) and its vector *Recilia dorsalis*, showed that the insect also uses the RNAi pathway to modulate virus infection (Lan *et al.*, 2016b). Silencing of Dicer-2 in this vector insect caused the virus to reach such high titres that the infection was lethal rather than persistent, which would preclude virus transmission (Lan *et al.*, 2016b). Therefore, a balance of virus infection in the vector must be maintained to allow for transmission.

Escape from the salivary glands is another crucial step in the transmission of circulative, propagative viruses. In *Nilaparvata lugens*, the vector of *Rice ragged stunt virus* (RRSV), virus infection appeared to induce apoptosis in the salivary gland of the vector (Fig. 6.2E), as visualized using a dUTP nick-end labelling assay (Huang *et al.*, 2015). Caspases are known to mediate apoptosis in animals, including insects. The authors searched the newly assembled *N. lugens* genome to identify caspases. Silencing of all copies of *Nlcaspase-1* prevented apoptosis in the salivary gland and diminished transmission of RRSV (Huang *et al.*, 2015). Therefore, inducing apoptosis in the vector salivary glands may be an important means for the virus to complete the transmission process (Huang *et al.*, 2015). Apoptosis may also be involved in transmission of CLas from *D. citri*, as enhanced levels of apoptosis are observed in adults insects reared on CLas-infected citrus trees (Ghanim *et al.*, 2016; Mann *et al.*, 2018).

Host manipulation by plant viruses: an indirect strategy for promoting vector transmission

Beyond the direct vector-pathogen protein interactions described above, successful virus transmission also relies on indirect effects on the vector and these effects have also been studied using 'omics methods. Over 50 years ago, Holmes and Bethel (1972) introduced the paradigm known as the 'host manipulation hypothesis' to explain the 'suicidal' behaviour displayed by parasitized animals that increased their risk of predation. From these observations, they proposed that pathogens evolve ways to control aspects of their host's behaviour to enhance their rate of transmission (reviewed in Heil, 2016). A most dramatic example of host manipulation is perhaps the *Ophiocordyceps unilateralis* fungus that infects *Camponotus leonardi* ants living in tropical rainforest trees. The infected ants, referred to as 'zombie' ants, adhere to very specific regions of vegetation using their mouth parts, which is critical for parasite fitness and dispersal (Andersen *et al.*, 2009).

It is well documented that insect-borne plant viruses and pathogens induce a myriad of adaptive visual and biochemical changes in host plants as infection progresses from the initial site of inoculation to full invasion of distal plant tissues (Pallas and Garcia, 2011). It was originally believed that disease symptoms such as chlorosis (yellowing of leaves) and inhibition of growth were simply deleterious side effects due to host resources being commandeered to support virus replication (Pallas and Garcia, 2011). However, numerous studies correlating plant pathology with insect vector performance and behaviour have since shown that most plant pathogens modify host physiology in adaptive ways that facilitate the type of host–vector relationship that favours their specific mode of transmission (Bosque-Pérez and Eigenbrode, 2011; Mann *et al.*, 2012; Mauck *et al.*, 2012; Gray *et al.*, 2014). Work with the citrus greening system has shown that effects of host manipulation extend beyond the ecology of plant virus–vector interactions: plant infection with the citrus greening bacterium induce

symptoms in plants which attract parasitoids of the insect vector (Martini *et al.*, 2014).

In general, beneficial effects on fitness and behaviour are observed when vectors are reared on plants infected with circulative pathogens whose dissemination is highly dependent on the prolonged feeding of one or a few vector species (Ajayi and Dewar, 1983; Castle and Berger, 1993; Castle *et al.*, 1998; Jiménez-Martíneza *et al.*, 2004; Maris *et al.*, 2004; Kotzampigikis *et al.*, 2010; Pelz-Stelinski and Killiny, 2016). For example, compared to virus-free hosts, the growth rate, longevity, and fecundity of the aphid *M. persicae* were enhanced when aphids were caged on potato plants infected with *Potato leafroll virus* (PLRV) (Fig. 6.2A), a circulative luteovirid that is primarily transmitted by this aphid species (Castle and Berger, 1993). For luteovirids, extension of feeding times increases the likelihood of vectors acquiring virus (Kotzampigikis *et al.*, 2010) while enhanced reproduction and increased alatae production creates a large reservoir of viruliferous insects that can carry and transmit virions to other plants (Gildow, 1980, 1983). In contrast, intermediate to no effects were observed when the same aphid species was reared on potato plants infected with the non-circulative virus, PVY, (Revers and Garcia, 2015), which requires only a brief interaction with aphids for transmission, or *Potato virus X* (PVX, *Potexvirus*), a vector-independent virus (Castle and Berger, 1993).

Vector manipulation by plant viruses promotes feeding behaviours aligned with the transmission mode that maximizes virus acquisition and transmission. When given a choice, non-viruliferous *M. persicae* prefer to immigrate and to settle on PLRV-infected leaves compared to those infected with PVX or PVY, or virus-free leaves (Castle *et al.*, 1998; Eigenbrode *et al.*, 2002). Over time, aphid emigration from PLRV-infected leaves is also reduced compared to the three other infection conditions (Eigenbrode *et al.*, 2002) and aphid-feeding behaviour becomes increased on older, symptomatic leaves (Alvarez *et al.*, 2007). However, as luteovirids are acquired, host preference switches and viruliferous aphids become more attracted to healthy plants (Medina-Ortega *et al.*, 2009) (Fig. 6.2A). For non-circulative viruses like CMV where acquisition occurs in seconds and retention is short-lived (Jacquemond, 2012), aphids are initially attracted to diseased plants, but dispersal occurs rapidly (Fig. 6.2A), indicating that these infected plants are quickly being perceived as poor-quality hosts (Mauck *et al.*, 2010a,b). Collectively, these studies along with other works testing additional plant-virus-vector systems (Ajayi and Dewar, 1983; Blua and Perring, 1992; Jiménez-Martíneza *et al.*, 2004; Maris *et al.*, 2004; Donaldson and Gratton, 2007; McMenemy *et al.*, 2012; Chen *et al.*, 2013; Maluta *et al.*, 2014; Wu *et al.*, 2014b; Claudel *et al.*, 2018) support Holmes and Bethel's hypothesis. However, exceptions in the literature can be found where effects on vectors are host specific (Castle *et al.*, 1998; Claudel *et al.*, 2018; DeBlasio *et al.*, 2018), neutral (Hodge and Powell, 2008), nuanced due to mixed infections (Salvaudon *et al.*, 2013; Lightle and Lee, 2014) or apparently contradictory to the host manipulation hypothesis (Blua *et al.*, 1994; Fiebig *et al.*, 2004; Boquel *et al.*, 2010; Casteel *et al.*, 2014). The application of 'omics technologies to identify the mechanisms and genetic features mediating these virus-induced effects on host–vector interactions has been instrumental in formulating a deeper understanding of plant virus epidemiology. Potential mechanisms proposed by these studies and details of the supporting data are discussed below.

The role of volatile cues in vector attraction

Vector attraction to infected plants has mainly been attributed to two factors: visual symptoms of disease such as leaf chlorosis, which play a general role in attracting insect vectors (Macias and Mink, 1969; Ajayi and Dewar, 1983; Holopainen *et al.*, 2009; Webster, 2012) and virus-induced changes to host volatile emissions that specifically influence insect responses (Eigenbrode *et al.*, 2002; Jiménez-Martíneza *et al.*, 2004; Ngumbi *et al.*, 2007; Medina-Ortega *et al.*, 2009; Werner *et al.*, 2009; Mauck *et al.*, 2010a; Rajabaskar *et al.*, 2013, 2014; Claudel *et al.*, 2018). Eigenbrode *et al.* (2002) were the first to show that non-viruliferous *M. persicae* were attracted to and preferentially arrested on filter paper models treated with headspace volatiles collected from PLRV-infected potatoes compared to those collected from healthy, PVY-, or PVX-infected plants, a result similar to what was observed when aphids were allowed to come in contact with infected leaves. In the context of a real infection, virus-induced changes to volatile cues are dynamic,

as aphids were more attracted to plants that were inoculated at a younger age compared to leaves that were more mature (Alvarez *et al.*, 2007; Werner *et al.*, 2009; Rajabaskar *et al.*, 2013). Metabolic profiling of headspace volatiles using quantitative gas chromatography coupled to MS (GC–MS) showed that six host compounds (limonene, pinene, cadinene, caryophyllene, α-humulene, and 7,11-dimethyl-3-methyl dodecatriene) of the 21 detected were elevated by PLRV-infection compared to the other virus infection treatments (Eigenbrode *et al.*, 2002) (Fig. 6.2A). Using a bead-free, quantitative affinity purification (AP)–MS workflow, which enables the identification of host protein interaction networks complexing with viruses, DeBlasio and colleagues have since shown that PLRV forms complexes *in planta* with the *N. benthamiana* protein α-humulene/(−)-(E)-b-caryophyllene synthase (DeBlasio *et al.*, 2017) (Fig. 6.2A). This enzyme is required for the biosynthesis of volatile sesquiterpenes including α-humulene (Tholl *et al.*, 2005), suggesting that PLRV influences the production of host volatile emissions at the protein level. Interestingly, only synthetic blends mimicking the exact concentration and composition of the naturally occurring PLRV-infected blend could elicit significant attraction/arrest of aphids compared to single applications of each compound and volatile blends from uninfected plants (Ngumbi *et al.*, 2007) indicating a more complex molecular mechanism may be at play. However, results from analysing other luteovirid-host-aphid systems favour the hypothesis that it may be the concentration of host volatiles emitted rather than metabolite composition that influences vector behaviour and that these changes are host-dependent (Jiménez-Martíneza *et al.*, 2004; Claudel *et al.*, 2018). Further experimentation looking at the effects that altering host and luteovirid protein expression/activity have on vector attraction as well as identifying the biochemical changes occurring within the insect in response to host volatile cues is needed to refine these predictions.

Selection has also favoured non-circulative viruses to have complex, multi-trophic mechanisms to ensure their plant-to-plant spread. For the non-circulative virus, CMV, Mauck *et al.* (2010a) demonstrated that, like circulative viruses, aphid vectors were initially attracted to the emissions of squash plants infected with the FNY strain compared to healthy hosts. However, aphid fitness was reduced, and insects quickly migrated away from these infected plants (Fig. 6.2A). Furthermore, volatile metabolites from infected plants were elevated in concentration but similar in composition to those produced by healthy plants. Thus, authors proposed that this strategy promotes CMV transmission by deceptively luring vectors to infected hosts with chemical cues that make the plant appear more appetizing from afar, while changes to plant quality (discussed below) facilitate immediate dispersal once virus is acquired by probing. A recent study has shown that volatiles emitted by CMV-infected tomato and *Arabidopsis thaliana*, such as pinene and p-cymene (Fig. 6.2A) allow for an additional evolutionary advantage by attracting host pollinators (Groen *et al.*, 2016). In choice experiments, bumble bees preferred CMV-infected plants to uninfected plants. Although CMV infection decreased seed yield in these hosts, enhanced fertilization due to increased interactions with bumble bees produced yields equivalent to uninfected plants. In addition, the bees could distinguish between volatiles emitted by plants infected with a CMV mutant unable to express the 2b RNA silencing suppressor and *A. thaliana* silencing mutants, implicating a role for host small RNA pathways in the production of virus-induced volatile emissions. Mathematical modelling showed that pollinator preference for virus-infected plants in the field could impart a selective advantage for the virus by allowing genes for disease susceptibility to persist in the population over host pathogen resistance (Groen *et al.*, 2016)

Virus-induced changes to the nutritional quality of hosts

Although volatile cues have been shown to play a major part in vector attraction and preference for infected hosts, data generated from metabolomic and proteomic profiling experiments have revealed that the nutritional quality of plant sap, the main food source for most insect vectors, is also significantly altered in ways that potentially make plants superior hosts when infected with circulative viruses (Bosque-Pérez and Eigenbrode, 2011) and poor ones when infected with non-circulative viruses (Mauck *et al.*, 2010a, 2014). Experiments with diet sachets have demonstrated that feeding behaviour and aphid fitness are positively influenced when the

levels of soluble sugars are higher in diet relative to amount of amino acids (Mittler, 1967; Puterka *et al.*, 2017). In wheat infected with the circulative BYDV, levels of soluble sugars, starch (Jensen, 1972) and the amino acids alanine and glutamine (Ajayi, 1986) were found to be elevated in symptomatic leaf tissue where chloroplast function was compromised (Fig. 6.2H). Indeed, positive effects on aphid survival and fitness can be observed when chloroplast function is severely disrupted through the down-regulation of *phytoene desaturase* (*PDS*), an essential enzyme in the biosynthesis of plastid-associated pigments (DeBlasio *et al.*, 2018). However, this effect was negated when aphids were reared for an extended time on *PDS*-silenced plants co-infected with PLRV, demonstrating that plants infected with circulative viruses eventually become poor-quality hosts, which would favour insect dispersal after virus is acquired. Characterization of host-virus protein interaction networks using quantitative AP-MS revealed that within host plants, PLRV proteins form complexes with host proteins that function in amino acid biosynthesis, carbohydrate metabolism, and photosynthesis (DeBlasio *et al.*, 2015b) (Fig. 6.2H). Interestingly, interactions between PLRV and a subset of these proteins are lost or weakened in the absence of the RTP, a PLRV structural protein known to facilitate symptom development (DeBlasio *et al.*, 2015c). Collectively these data suggest that, like the regulation of host volatile emissions, PLRV capsid proteins may function to change the composition of plant sap by binding to plant proteins involved in nutrient metabolism and altering or redirecting their activity.

Studies evaluating the nutritional quality of host plants infected with the non-circulative virus CMV-FNY showed the ratio of carbohydrates to amino acids to be reduced in phloem and non-vascular cells of infected squash compared to healthy plants (Mauck *et al.*, 2014). These infected plants also had lower levels of the essential amino acids that are required for aphid survival (Mauck *et al.*, 2014). Thus, CMV-infection effectively creates an antagonistic feeding environment that could potentially promote vector dispersal. Electrical penetration graph measurements show that aphids do ingest less phloem sap on CMV-infected *A. thaliana* plants (Westwood *et al.*, 2013). CMV-induced deterrence to aphid feeding in *A. thaliana* is regulated by the interplay between CMV 2b and two other viral proteins (1a and 2a) and their effects on host Argonaute 1 (Westwood *et al.*, 2013). However, these effects are most likely host-dependent as CMV-FNY infection leads to an increase of soluble sugars in melon phloem sap (Shalitin and Wolf, 2000) and positive effects on aphid performance in tobacco plants, which has also been shown to be regulated by the CMV 2b-silencing suppressor (Ziebell *et al.*, 2011).

Alteration of plant defenses to insects

Manipulation of host defense against the insect is yet another way plant viruses can indirectly influence vector performance and behaviour. In the absence of virus, plants respond to herbivore attack through the up-regulation of an array of host defensive molecules that decrease insect fitness and deter feeding (War *et al.*, 2012). Global profiling of mRNA and protein expression in infected hosts show that plant viruses work to either suppress or activate host pathways regulating these responses (Whitham *et al.*, 2006; Di Carli *et al.*, 2012). In general, the phytohormones jasmonic acid (JA) and ethylene (ET) act as anti-herbivore signalling molecules (Morkunas *et al.*, 2011). In CMV-infected squash plants where negative impacts on aphid performance have been observed, significantly higher levels of JA are induced by aphid feeding compared to observations on healthy plants (Fig. 6.2H). Ethylene emissions were also increased in infected plants (Mauck *et al.*, 2014) (Fig. 6.2H). Microarray analysis of CMV-infected *A. thaliana* showed up-regulation of a gene coding for an enzyme involved in the biosynthesis of 4-methoxy-indol-3-yl-methylglucosinolate, a known aphid deterrent (Westwood *et al.*, 2013) (Fig. 6.2H). These effects are consistent with favouring the non-circulative mode of transmission where insect deterrence would be advantageous. In contrast, ET signalling is required for *Turnip mosaic virus* (TuMV, *Potyvirus*) suppression of callose deposition against *M. persicae*, which leads to a positive effect on aphid performance (Casteel *et al.*, 2014, 2015). In an elegant demonstration of the tritrophic interaction between plant, virus, and insect, this process was found to be mediated by the aphid-induced localization of the potyvirus Nuclear Inclusion a-Protease (NIa-Pro) to the plant vacuole showing that potyviruses manipulate their

host plant into promoting vector performance only when their vector is present and the response is needed (Bak *et al.*, 2017).

Circulative viruses manipulate plant host physiology in ways that impact plant defense against the vector. For the begomovirus *Tomato yellow leaf curl China virus* (TYLCCNV), which is transmitted in a circulative manner by whiteflies, co-infection of tobacco with its betasatellite represses host JA-mediated responses against the insect (Zhang *et al.*, 2012). Specifically, the betasatellite encoded βC1 protein represses the expression of host JA-biosynthesis and JA-regulated genes (Fig. 6.2H), which leads to a decrease in JA production and an increase in vector fitness. Although information is lacking on the global effects luteovirid infection has on plant defense and how that relates to vector performance, AP-MS experiments show that host proteins with functions in host defense, including callose deposition and JA biosynthesis (i.e. lipoxygenases), complex *in planta* with PLRV proteins (DeBlasio *et al.*, 2015b,c, 2017).

Changes induced within the vector

Although an extensive amount of work has been done to understand how viruses modify plants, scientists are now starting to focus on characterizing the changes within the insect that occur due to ingestion/perception of virus-induced host plant signals. There have been some studies comparing the transcription profiles of insect vectors fed on virus-infected plants compared to healthy hosts (Brault *et al.*, 2010; Zhang *et al.*, 2010; Cassone *et al.*, 2014). However, there are few reports where the effects of infected host on vector biochemistry can be clearly separated from the direct effects of virus (Brizard *et al.*, 2006; Bencharki *et al.*, 2010; Cilia *et al.*, 2012b; Pinheiro *et al.*, 2017). Proteomics has been used to identify interactions between host and virus that are required for acquisition and transmission (Brizard *et al.*, 2006; Bencharki *et al.*, 2010; Cilia *et al.*, 2012b). Treatment of the purified luteovirid CYDV with sodium sulfite eliminates its ability to be transmitted by its aphid vector *R. padi*, even though virion morphology was unaffected (Cilia *et al.*, 2012b). Analysis of virus preparations by nanoscale liquid chromatography tandem MS (nLC-MS/MS) revealed host plant proteins co-purifying with transmissible virion that were lost when CYDV was treated with sodium sulfite, suggesting that these host–virus interactions may be critical for virus uptake. Targeted MS analysis of aphids fed on CYDV-infected plants showed that several of these CYDV-associated host proteins accumulated to higher levels in the insect compared to those fed on healthy plants (Cilia *et al.*, 2012b), consistent with a previous observation that addition of high quantities of soluble host proteins to diet increases the transmission of a related polerovirus (Bencharki *et al.*, 2010). Yet, in both of these reports, the cellular and biochemical effects these plant proteins have on the vector were never assessed. Recently, Pinheiro *et al.* (2017) found that the circulative transmission of PLRV is reduced when *M. persicae* is reared on turnip plants, a host for the aphid but a non-host for the virus. Using a combination of proteomic, biochemical, and microscopic approaches, they demonstrated that signals derived from the non-host plant inhibited PLRV transmission by altering the activity and localization of the aphid cysteine protease cathepsin B within gut cells (Fig. 6.2C). It is possible that up-regulation of cathepsin B in the aphid gut degrades host proteins needed for successful virus acquisition, thus, having an inhibitory effect on virus transmission.

Vector manipulation by plant viruses: a direct strategy for promoting vector transmission

The vector manipulation hypothesis was described as recently as 2012 in a landmark paper by Ingwell *et al.* (2012). The authors observed that while non-viruliferous *R. padi* aphids were attracted to plants infected with the luteovirid BYDV, once the aphids acquired virus, their preference switched to healthy, uninfected plants, a behaviour change which would promote transmission (Ingwell *et al.*, 2012). Therefore, the authors described the vector manipulation hypothesis as 'the evolution of strategies in plant pathogens to enhance their spread to new hosts.' This hypothesis describes direct effects of the virus on the vector and is distinguished from the host manipulation hypothesis, which deals with indirect effects on the vector mediated by an infected plant host. To observe direct effects of a plant virus on its insect vector, insects must be removed from the infected plant to allow gut clearing of non-acquired virus and transient signals from the infected plant.

This is accomplished either by having insects acquire purified virus in a membrane feeding setup as in the BYDV study (Ingwell *et al.*, 2012), and/or by moving insects to a non-host plant after acquisition (Pinheiro *et al.*, 2017). This section describes novel insights gained into the evolutionary interactions involved in transmission by observing the direct effects of virus on vector behaviour/performance and leveraging 'omics techniques.

Insights from viruses transmitted in a circulative, non-propagative mode

In a follow-up to the 2012 study with BYDV (Ingwell *et al.*, 2012), the same research group examined the preferences of *M. persicae* in response to PLRV, another luteovirid transmitted in the circulative, non-propagative mode (Rajabaskar *et al.*, 2014). Consistent with their previous findings, the authors found that non-viruliferous aphids preferentially settle on PLRV-infected plants, whereas viruliferous *M. persicae* preferred mock-inoculated potato plants (Rajabaskar *et al.*, 2014). Using GC-MS, the authors identified many of the volatile organic compounds produced by healthy and PLRV-infected plants. Similar to the work of Eigenbrode *et al.* (2002) with non-viruliferous *M. persicae*, Rajabaskar *et al.* (2014) repeated the choice assays with non-viruliferous and viruliferous aphids using only trapped plant headspace volatiles or synthetic volatile blends mimicking healthy and infected plants. Even in the absence of actual potato plants, the authors still observed the same aphid preferences, showing that these olfactory cues alone were enough to determine aphid host selection.

The interactions between begomoviruses and their whitefly vectors are often more akin to a pathogen–host relationship rather than a non-propagative virus–vector one. TYLCV reduces the life expectancy and fecundity of its vector *B. tabaci* (Rubinstein and Czosnek, 1997). In transcriptome analysis of TYLCCNV-viruliferous whiteflies, the virus was shown to alter genes related to the cell cycle and primary metabolism, which may explain the reductions in insect longevity and fecundity (Luan *et al.*, 2011). Additionally, whitefly immune responses such as autophagy and antimicrobial peptide production were activated in response to TYLCCNV (Luan *et al.*, 2011). Transcriptome profiling of dissected guts of TYLCV-viruliferous whiteflies also showed perturbation of the cell cycle and induction of defense, including antimicrobial peptides (Geng *et al.*, 2018). In electrical penetration graph feeding experiments, TYLCV-viruliferous whiteflies fed more readily than their non-viruliferous counterparts and spent more time salivating into the phloem, which is key for virus transmission (Liu *et al.*, 2013). A similar study with TYLCCNV found distinct differences in feeding behaviour of viruliferous *B. tabaci* when feeding on a host or non-host of the virus (He *et al.*, 2015). The virus inhibited whitefly feeding on cotton, a non-host, and on a resistant cultivar of tobacco, but improved whitefly feeding on TYLCCNV-infected tobacco (He *et al.*, 2015). Therefore, this improvement in feeding may be an indirect effect mediated by the infected plant rather than a direct effect.

Insights from viruses transmitted in a circulative, propagative mode

Recent work in the RSV-small brown planthopper system, guided by transcriptomics data, delved into the two common phenotypes of vector manipulation: attraction to infected plant volatiles and alteration of vector fecundity. While several studies have shown that volatiles produced by virus-infected plants may help attract insect vector species (reviewed above) little work has been done on the vector side of this interaction. In a functional study from the RSV system, a key olfactory receptor was identified in the small brown planthopper (Li *et al.*, 2019). Using the *L. striatellus* transcriptome (Zhang *et al.*, 2010) and a known sequence of the olfactory co-receptor Orco from the closely related *Nilaparvata lugens*, the authors confirmed the existence of a homologue of Orco in the small brown planthopper. Following orally delivered dsRNA-mediated knock-down of the *Orco* gene in *L. striatellus*, the insects were given the choice between a healthy rice plant or open air. After silencing of *Orco*, the response time of the insects was greatly increased, and a larger proportion presented no response or chose the air treatment. This finding confirms the role of Orco in olfactory host-seeking behaviour. The authors also found that RSV-viruliferous planthoppers had higher *Orco* expression, and present stronger host-seeking behaviour as indicated by a lower proportion of insects with no response in the choice assay. These findings indicate that the spread of RSV may be increased by the up-regulation of an

olfactory receptor in the RSV vector, which would aid the planthopper in seeking out host plants.

There is a lack of consensus in the literature regarding how RSV affects the fecundity of its vector, but fecundity manipulation may be important for virus epidemiology. Li *et al.* (2015) observed a decrease in hatchability of planthopper eggs when one or more parent was viruliferous with RSV, consistent with a previous study (Nasu, 1963). The authors assessed the expression of 115 genes related to embryonic development, which they chose by harnessing the power of *D. melanogaster* as a model for insect developmental biology and mining homologous genes from *L. striatellus* (Zhang *et al.*, 2010). They attributed the decrease in hatchability and correlated egg defects to significant down-regulation of two important embryonic development genes: *Ls-Dorsal*, a transcription factor regulating tissue differentiation and *Ls-CPO*, an enzyme responsible for crosslinking and hardening of the protective chorion surrounding eggs (Fig. 6.2D). In contrast, Wan *et al.* observed no significant difference in hatching between RSV-viruliferous and non-viruliferous planthoppers but instead observed a decreased number of eggs laid by viruliferous females (Wan *et al.*, 2015). These authors tied this observation to decreased expression of *Vg* in viruliferous-females, which is a key yolk protein necessary for both egg development and the passage of RSV into nurse cells for transovarial transmission (Huo *et al.*, 2014). Furthermore, Wan *et al.* observed accelerated nymphal development, attributed to down-regulation of *JHMAT* in the juvenile hormone (JH) pathway, and up-regulation of *CYP307A1* in the ecdysteroid pathway (Fig. 6.2B). This expression pattern is consistent with immunosuppression by RSV, as JH serves as an immune activator and 20-hydroxy-ecdysone (an ecdysteroid) acts as an inhibitor in other insect systems (Beckstead *et al.*, 2005; Tian *et al.*, 2010). Despite these conflicting findings, RSV has a major role in altering embryogenesis and development of its insect vector. Work by He and colleagues suggests that fecundity manipulation may be a beneficial strategy for the virus to spread within a crop, as the proportion of viruliferous insects in the population rather than the absolute population size of the small brown plant-hopper vector is positively correlated with disease severity in rice fields in China (He *et al.*, 2016).

Vector manipulation has also been observed in plant-infecting reoviruses. In a compelling parallel to the studies performed on the luteovirid system, Wang, H. *et al.* (2014) showed that while non-viruliferous *S. furcifera* prefer SRBSDV-infected plants, SRBSDV-viruliferous insects prefer uninfected plants, which would promote virus dispersal by viruliferous individuals seeking new susceptible host plants (Wang, H. *et al.*, 2014). These authors also looked at the preferences of planthoppers given an acquisition access period on SRBSDV-infected plants, which did not become viruliferous. Intriguingly, these exposed, nonviruliferous *S. furcifera* preferred uninfected rice plants over SRBSDV-infected ones, which is inconsistent with the vector manipulation hypothesis. As SRBSDV-infected plants have adverse effects on vector fecundity, development, and longevity (Tu *et al.*, 2013), the authors propose that the planthoppers 'remember' the unfavourable quality of these infected plants, as well as their odour, and are subsequently deterred when exposed to the same volatile cues, although these same insects preferred the odour of an infected plant to no plant (air). In a final twist of this experiment, the authors looked at the host preference of a non-vector of SRBSDV, *N. lugens*, the brown planthopper, and vector of RRSV, another reovirus that commonly co-infects plants with SRBSDV. *N. lugens* preferred uninfected rice plants to SRBSDV-infected plants, consistent with previous studies showing a virus-infected plant is often unattractive to non-vector species (van Molken *et al.*, 2012; Mauck *et al.*, 2014). However, after *N. lugens* acquired RRSV, its preference switched from healthy rice plants to SRBSDV-infected ones. The authors hypothesize that either RRSV causes this change in its vector's behaviour, or SRBSDV is altering the behaviour of a non-vector species. Either way, this manipulation favours co-infection of the two viruses, which are commonly found in mixed infections.

One relatively unexplored area of vector manipulation is response to abiotic stress. Xu *et al.* (2016) looked at the thermal tolerance of SRBSDV-viruliferous planthoppers under extreme heat and cold conditions, as well as performing transcriptomics on insects exposed to virus and/or extreme temperature. They found that viruliferous insects were better able to tolerate extreme heat stress, which has important implications for the spread of

SRBSDV epidemics in the summer. Additionally, viruliferous insects experienced higher mortality under cold stress, which may affect the ability of planthoppers to overwinter. The transcriptomics data revealed general up-regulation of expression in viruliferous insects exposed to cold stress and down-regulation of transcription in those exposed to extreme heat. The authors observed nuanced expression of heat shock proteins in insects exposed to SRBSDV and heat stress, which may be due to the crosstalk between heat stress and antiviral pathways, both of which would be activated under this dual stress and potentially repressed by viral counter-defense strategies. In a follow-up metabolomics study of *S. furcifera* exposed to the same virus and environmental stressors, there was up-regulation of polyols and sugars in viruliferous planthoppers under both heat and cold stress, and up-regulation of certain amino acids, such as methionine, proline, threonine, ornithine, L-homoserine and β-alanine, under heat stress (Zhang *et al.*, 2018). These same amino acids were down-regulated in response to cold stress. Therefore, the virus may alter the insect's abiotic stress response, which could impact virus and vector seasonal migration, especially in the face of climate change.

Future directions

Rapid advances in 'omics technologies include instruments used for detection with increased sensitivity being built more quickly and cheaply. The advent of recent technologies such as single-cell transcriptomics (Ziegenhain *et al.*, 2018), laser-capture microdissection (Zhu *et al.*, 2016) and MALDI-imaging (Vrkoslav *et al.*, 2010), which can be used to detect RNA, protein, or metabolite species (respectively) within a small population of cells or an individual cell, has allowed for the generation of expression atlas resources for model insect species that detail tissue specific expression patterns/responses that would have been lost in studies using whole insects.

As more genomic, proteomic, and metabolomic information becomes available for non-model vector species, systems-wide studies comparing 'omics datasets across different virus-vector systems is needed to determine what is generally involved in plant pathogen transmission by insects and what is specific. In the literature, many of the same vector proteins are reported to be involved in the transmission process for diverse virus groups, indicating potential conservation or co-evolution of viruses to co-opt these conserved proteins during transmission. These same proteins may also be involved in the ability of insect vectors to rapidly adapt and colonize new host plants, as expanded gene families for specific sets of proteins in aphids, including cuticular proteins and cathepsin B, are also involved in host adaptation (Mathers *et al.*, 2017). However, considering genome annotations are largely derived from homology to distant species (Saha *et al.*, 2017), it is difficult to know to what extent 'omics methods are biased towards these conserved proteins and are currently unable to identify species-specific genes and proteins that may play crucial roles in transmission or other aspects of vector biology. Future genome annotation efforts should seek to overcome these limitations. Improving RNAi techniques in non-model insects can aid functional validation of annotations.

Many of the 'omics techniques discussed can be applied to understanding the biology and transmission of insect-infecting viruses, which have the potential for use as RNAi delivery systems (Heck, 2018). RNA sequencing techniques are being used to discover novel insect-infecting viruses (reviewed in Nouri *et al.*, 2018; see also Chapter 1), and the development of infectious clones can aid reverse genetic approaches to understand the function of viral proteins. Transcriptomics and proteomics can be used to discover insect proteins involved in transmission of these insect viruses, as described in this chapter for plant viruses, and functional genomics, such as dsRNA feeding, can be used to validate the function of insect proteins in some cases.

Insect-infecting viruses may be an undescribed partner in the transmission of plant viruses. A compelling paper by Pinheiro *et al.* (2019) provides a fascinating example. Using small RNA sequencing, the authors discovered that plants infected with the luteovirid PLRV alter the production of small RNAs (sRNAs) in the aphid vector, *M. persicae*, producing unusually large-sized RNAs matching to *Myzus persicae densovirus* (MpDNV). Aphids viruliferous with PLRV displayed higher titres of MpDNV, suggesting these aberrant sRNA sizes are a reflection of an altered anti-virus defense response in the aphid. Densoviruses are ubiquitous among arthropods (Fédière, 2000), and have even

been shown to promote wing morph development in aphids, which are polyphenic and produce both winged and non-winged individuals (Ryabov *et al.*, 2009). Pinheiro *et al.* (2019) hypothesize that PLRV suppresses the immune system of its aphid vector to allow for the proliferation of MpDNV, an insect-infecting virus that promotes the development of more winged individuals, which in turn would increase plant-to-plant spread of PLRV.

In addition to the impact of insect-infecting viruses on transmission, vector endosymbionts are other crucial biological players that may affect virus transmission indirectly. Many economically important insect vectors, such aphids and whiteflies, harbour obligate and secondary endosymbionts. The role of endosymbionts in the transmission of plant viruses has been debated and reviewed elsewhere (Pinheiro *et al.*, 2015). Manipulation of endosymbionts may prove to be an important frontier in developing novel control strategies against insect-transmitted plant viruses, as successfully shown in animal-infecting arboviruses (Durvasula *et al.*, 2003; Hoffmann *et al.*, 2011).

Finally, these compelling insights into the mechanisms of transmission in vector biology in recent years need to be channelled into the development of control strategies. As more receptors for virus acquisition in insects become known, strategies can be developed to block or reduce virus binding to these crucial proteins, either through down-regulation of the receptor in the insect by RNAi (Liang and Gao, 2017; Mulot *et al.*, 2018; Webster *et al.*, 2018), or through the application of chemical or peptide inhibitors (Liu *et al.*, 2010). Other control strategies tailored to the biology of the system in question, such as decreasing the susceptibility of vectors of circulative, propagative viruses so that virus titres do not reach levels conducive for transmission in the vector, an approach that has been deployed for mosquito-borne arboviruses (Hoffmann *et al.*, 2011), should also be explored. Conversely, considering the finding that insect sRNA-mediated antiviral immunity can keep circulative, propagative virus populations in check (Lan *et al.*, 2016a,b), an alternate strategy may be to suppress the antiviral defenses of the vector, such that virus titres reach a level that kill vectors before transmission can occur. As newer and more powerful technologies are developed, our understanding of the biology of these insect-transmitted plant viruses will only increase, as should development and deployment of more effective control strategies.

Funding

The authors are funded by NSF grant 1354309 (to MH), USDA-ARS CRIS Project 8062-22410-006-00-D (to MH) and an NSF Graduate Research Fellowship DGE-1650441 (to JRW).

References

Ajayi, O. (1986). The effect of *Barley yellow dwarf virus* on the amino acid composition of spring wheat. Ann. Appl. Biol. *108*, 145–149.

Ajayi, O., and Dewar, A.M. (1983). The effect of *Barley yellow dwarf virus* on field populations of the cereal aphids, *Sitobion avenae* and *Metopolophium dirhodum*. Ann. Appl. Biol. *103*, 1–11.

Alexander, M.M., Mohr, J.P., DeBlasio, S.L., Chavez, J.D., Ziegler-Graff, V., Brault, V., Bruce, J.E., and Heck, M.C. (2017). Insights in luteovirid structural biology guided by chemical cross-linking and high resolution mass spectrometry. Virus Res. *241*, 42–52. https://doi.org/10.1016/j.virusres.2017.05.005

Allison, R.F., Dougherty, W.G., Parks, T.D., Willis, L., Johnston, R.E., Kelly, M., and Armstrong, F.B. (1985). Biochemical analysis of the capsid protein gene and capsid protein of *Tobacco etch virus*: N-terminal amino acids are located on the virion's surface. J. Virol. *147*, 309–316. https://doi.org/10.1016/0042-6822(85)90133-3

Alvarez, A.E., Garzo, E., Verbeek, M., Vosman, B., Dicke, M., and Tjallingii, W.F. (2007). Infection of potato plants with *Potato leafroll virus* changes attraction and feeding behaviour of *Myzus persicae*. Entomol. Exp. Appl. *125*, 135–144. https://doi.org/10.1111/j.1570-7458.2007.00607.x

Ammar, E.D., Jarlfors, U., and Pirone, T.P. (1994). Association of potyvirus helper component protein with virions and the cuticle lining the maxillary food canal and foregut of an aphid vector. Phytopathology *84*, 1054–1060.

Andersen, S.B., Gerritsma, S., Yusah, K.M., Mayntz, D., Hywel-Jones, N.L., Billen, J., Boomsma, J.J., and Hughes, D.P. (2009). The life of a dead ant: the expression of an adaptive extended phenotype. Am. Nat. *174*, 424–433. https://doi.org/10.1086/603640

Artimo, P., Jonnalagedda, M., Arnold, K., Baratin, D., Csardi, G., de Castro, E., Duvaud, S., Flegel, V., Fortier, A., Gasteiger, E., *et al.* (2012). ExPASy: SIB bioinformatics resource portal. Nucleic Acids Res. *40*, W597–603. https://doi.org/10.1093/nar/gks400

Atreya, P.L., Atreya, C.D., and Pirone, T.P. (1991). Amino acid substitutions in the coat protein result in loss of insect transmissibility of a plant virus. Proc. Natl. Acad. Sci. U.S.A. *88*, 7887–7891.

Atreya, P.L., Lopez-Moya, J.J., Chu, M., Atreya, C.D., and Pirone, T.P. (1995). Mutational analysis of the coat protein N-terminal amino acids involved in potyvirus

transmission by aphids. J. Gen. Virol. *76*, 265–270. https://doi.org/10.1099/0022-1317-76-2-265

Azzam, O., Frazer, J., de la Rosa, D., Beaver, J.S., Ahlquist, P., and Maxwell, D.P. (1994). Whitefly transmission and efficient ssDNA accumulation of *Bean golden mosaic* geminivirus require functional coat protein. Virology *204*, 289–296.

Badillo-Vargas, I.E., Rotenberg, D., Schneweis, D.J., Hiromasa, Y., Tomich, J.M., and Whitfield, A.E. (2012). Proteomic analysis of *Frankliniella occidentalis* and differentially expressed proteins in response to *Tomato spotted wilt virus* infection. J. Virol. *86*, 8793–8809. https://doi.org/10.1128/JVI.00285-12

Badillo-Vargas, I.E., Chen, Y., Martin, K.M., Rotenberg, D., and Whitfield, A.E. (2018). Discovery of novel thrips vector proteins that bind to the plant bunyavirus, *Tomato spotted wilt virus*. bioRxiv 416560 (preprint). https://doi.org/10.1101/416560

Bak, A., Gargani, D., Macia, J.L., Malouvet, E., Vernerey, M.S., Blanc, S., and Drucker, M. (2013). Virus factories of *Cauliflower mosaic virus* are virion reservoirs that engage actively in vector transmission. J. Virol. *87*, 12207–12215. https://doi.org/10.1128/JVI.01883-13

Bak, A., Cheung, A.L., Yang, C., Whitham, S.A., and Casteel, C.L. (2017). A viral protease relocalizes in the presence of the vector to promote vector performance. Nat. Commun. *8*, 14493. https://doi.org/10.1038/ncomms14493

Bandla, M.D., Campbell, L.R., Ullman, D.E., and Sherwood, J.L. (1998). Interaction of *Tomato spotted wilt* tospovirus (TSWV) glycoproteins with a Thrips midgut protein, a potential cellular receptor for TSWV. Phytopathology *88*, 98–104. https://doi.org/10.1094/PHYTO.1998.88.2.98

Beckstead, R.B., Lam, G., and Thummel, C.S. (2005). The genomic response to 20-hydroxyecdysone at the onset of *Drosophila* metamorphosis. Genome Biol. *6*, R99.

Bencharki, B., Boissinot, S., Revollon, S., Ziegler-Graff, V., Erdinger, M., Wiss, L., Dinant, S., Renard, D., Beuve, M., Lemaitre-Guillier, C., *et al.* (2010). Phloem protein partners of *Cucurbit aphid borne yellows virus*: possible involvement of phloem proteins in virus transmission by aphids. Mol. Plant Microbe Interact. *23*, 799–810. https://doi.org/10.1094/MPMI-23-6-0799

Berlandier, F., Thackray, D., Jones, R., Latham, L., and Cartwright, L. (1997). Determining the relative roles of different aphid species as vectors of *Cucumber mosaic* and *Bean yellow mosaic* viruses in lupins. Ann. Appl. Biol. *131*, 297–314. https://doi.org/10.1111/j.1744-7348.1997.tb05158.x

Blua, M.J., and Perring, T.M. (1992). Effects of *Zucchini yellow mosaic virus* on coloization and feeding behavior of *Aphis gossypii* (Homoptera: Aphididae) alatae. Environ. Entomol. *21*, 578–585. https://doi.org/10.1093/ee/21.3.578

Blua, M.J., Perring, T.M., and Madore, M.A. (1994). Plant virus-induced changes in aphid population development and temporal fluctuations in plant nutrients. J. Chem. Ecol. *20*, 691–707. https://doi.org/10.1007/BF02059607

Boissinot, S., Erdinger, M., Monsion, B., Ziegler-Graff, V., and Brault, V. (2014). Both structural and non-structural forms of the readthrough protein of *Cucurbit aphid-borne yellows virus* are essential for efficient systemic infection of plants. PLOS ONE *9*, e93448. https://doi.org/10.1371/journal.pone.0093448

Boquel, S., Giordanengo, P., and Ameline, A. (2010). Divergent effects of PVY-infected potato plant on aphids. Eur. J. Plant Pathol. *129*, 507–510. https://doi.org/10.1007/s10658-010-9732-8

Bosque-Pérez, N.A., and Eigenbrode, S.D. (2011). The influence of virus-induced changes in plants on aphid vectors: insights from luteovirus pathosystems. Virus Res. *159*, 201–205. https://doi.org/10.1016/j.virusres.2011.04.020

Bouvaine, S., Boonham, N., and Douglas, A.E. (2011). Interactions between a luteovirus and the GroEL chaperonin protein of the symbiotic bacterium *Buchnera aphidicola* of aphids. J. Gen. Virol. *92*, 1467–1474. https://doi.org/10.1099/vir.0.029355-0

Brault, V., Mutterer, J., Scheidecker, D., Simonis, M.T., Herrbach, E., Richards, K., and Ziegler-Graff, V. (2000). Effects of point mutations in the readthrough domain of the *Beet western yellows virus* minor capsid protein on virus accumulation in planta and on transmission by aphids. J. Virol. *74*, 1140–1148. https://doi.org/10.1128/JVI.74.3.1140-1148.2000

Brault, V., Bergdoll, M., Mutterer, J., Prasad, V., Pfeffer, S., Erdinger, M., Richards, K.E., and Ziegler-Graff, V. (2003). Effects of point mutations in the major capsid protein of *Beet western yellows virus* on capsid formation, virus accumulation, and aphid transmission. J. Virol. *77*, 3247–3256. https://doi.org/10.1128/JVI.77.5.3247-3256.2003

Brault, V., Périgon, S., Reinbold, C., Erdinger, M., Scheidecker, D., Herrbach, E., Richards, K., and Ziegler-Graff, V. (2005). The polerovirus minor capsid protein determines vector specificity and intestinal tropism in the aphid. J. Virol. *79*, 9685–9693. https://doi.org/10.1128/JVI.79.15.9685-9693.2005

Brault, V., Tanguy, S., Reinbold, C., Le Trionnaire, G., Arneodo, J., Jaubert-Possamai, S., Guernec, G., and Tagu, D. (2010). Transcriptomic analysis of intestinal genes following acquisition of *Pea enation mosaic virus* by the pea aphid *Acyrthosiphon pisum*. J. Gen. Virol. *91*, 802–808. https://doi.org/10.1099/vir.0.012856-0

Bressan, A., and Watanabe, S. (2011). Immunofluorescence localisation of *Banana bunchy top virus* (family *Nanoviridae*) within the aphid vector, *Pentalonia nigronervosa*, suggests a virus tropism distinct from aphid-transmitted luteoviruses. Virus Res. *155*, 520–525. https://doi.org/10.1016/j.virusres.2010.12.005

Briddon, R.W., Pinner, M.S., Stanley, J., and Markham, P.G. (1990). Geminivirus coat protein gene replacement alters insect specificity. Virology *177*, 85–94. https://doi.org/10.1016/0042-6822(90)90462-z

Brizard, J.P., Carapito, C., Delalande, F., Van Dorsselaer, A., and Brugidou, C. (2006). Proteome analysis of plant-virus interactome: comprehensive data for virus multiplication inside their hosts. Mol. Cell Proteomics *5*, 2279–2297. https://doi.org/10.1074/mcp.M600173-MCP200

Caciagli, P., Medina Piles, V., Marian, D., Vecchiati, M., Masenga, V., Mason, G., Falcioni, T., and Noris, E. (2009). Virion stability is important for the circulative transmission of *Tomato yellow leaf curl sardinia virus* by

Bemisia tabaci, but virion access to salivary glands does not guarantee transmissibility. J. Virol. *83*, 5784–5795. https://doi.org/10.1128/JVI.02267-08

Cassone, B.J., Wijeratne, S., Michel, A.P., Stewart, L.R., Chen, Y., Yan, P., and Redinbaugh, M.G. (2014). Virus-independent and common transcriptome responses of leafhopper vectors feeding on maize infected with semi-persistently and persistent propagatively transmitted viruses. BMC Genomics *15*, 133. https://doi.org/10.1186/1471-2164-15-133

Casteel, C.L., Yang, C., Nanduri, A.C., De Jong, H.N., Whitham, S.A., and Jander, G. (2014). The NIa-Pro protein of *Turnip mosaic virus* improves growth and reproduction of the aphid vector, *Myzus persicae* (green peach aphid). Plant J. *77*, 653–663. https://doi.org/10.1111/tpj.12417

Casteel, C.L., De Alwis, M., Bak, A., Dong, H., Whitham, S.A., and Jander, G. (2015). Disruption of ethylene responses by *Turnip mosaic virus* mediates suppression of plant defense against the green peach aphid vector. Plant Physiol. *169*, 209–218. https://doi.org/10.1104/pp.15.00332

Castle, S.J., and Berger, P.H. (1993). Rates of growth and increase of *Myzus persicae* on virus-infected potatoes according to type of virus-vector relationship. Entomol. Exp. Appl. *69*, 51–60. https://doi.org/10.1111/j.1570-7458.1993.tb01727.x

Castle, S.J., Mowry, T.M., and Berger, P.H. (1998). Differential settling by *Myzus persicae* (Homoptera: Aphididae) on various virus infected host plants. Ann. Entomol. Soc. Am. *91*, 661–667. https://doi.org/10.1093/aesa/91.5.661

Chavez, J.D., Cilia, M., Weisbrod, C.R., Ju, H.J., Eng, J.K., Gray, S.M., and Bruce, J.E. (2012). Cross-linking measurements of the *Potato leafroll virus* reveal protein interaction topologies required for virion stability, aphid transmission, and virus–plant interactions. J. Proteome Res. *11*, 2968–2981. https://doi.org/10.1021/pr300041t

Chay, C.A., Gunasinge, U.B., Dinesh-Kumar, S.P., Miller, W.A., and Gray, S.M. (1996). Aphid transmission and systemic plant infection determinants of *Barley yellow dwarf luteovirus-PAV* are contained in the coat protein readthrough domain and 17-kDa protein, respectively. Virology *219*, 57–65. https://doi.org/10.1006/viro.1996.0222

Chen, G., Pan, H., Xie, W., Wang, S., Wu, Q., Fang, Y., Shi, X., and Zhang, Y. (2013). Virus infection of a weed increases vector attraction to and vector fitness on the weed. Sci. Rep. *3*, 2253. https://doi.org/10.1038/srep02253

Chen, W., Hasegawa, D.K., Kaur, N., Kliot, A., Pinheiro, P.V., Luan, J., Stensmyr, M.C., Zheng, Y., Liu, W., Sun, H., *et al.* (2016). The draft genome of whitefly *Bemisia tabaci* MEAM1, a global crop pest, provides novel insights into virus transmission, host adaptation, and insecticide resistance. BMC Biol. *14*, 110. https://doi.org/10.1186/s12915-016-0321-y

Chen, Y., Cassone, B.J., Bai, X., Redinbaugh, M.G., and Michel, A.P. (2012). Transcriptome of the plant virus vector *Graminella nigrifrons*, and the molecular interactions of *Maize fine streak* rhabdovirus transmission. PLOS ONE *7*, e40613. https://doi.org/10.1371/journal.pone.0040613

Christodoulides, N., Van Dam, A.R., Peterson, D.A., Frandsen, R.J.N., Mortensen, U.H., Petersen, B., Rasmussen, S., Normark, B.B., and Hardy, N.B. (2017). Gene expression plasticity across hosts of an invasive scale insect species. PLOS ONE *12*, e0176956. https://doi.org/10.1371/journal.pone.0176956

Cilia, M., Tamborindeguy, C., Fish, T., Howe, K., Thannhauser, T.W., and Gray, S. (2011a). Genetics coupled to quantitative intact proteomics links heritable aphid and endosymbiont protein expression to circulative polerovirus transmission. J. Virol. *85*, 2148–2166. https://doi.org/10.1128/JVI.01504-10

Cilia, M., Tamborindeguy, C., Rolland, M., Howe, K., Thannhauser, T.W., and Gray, S. (2011b). Tangible benefits of the aphid *Acyrthosiphon pisum* genome sequencing for aphid proteomics: Enhancements in protein identification and data validation for homology-based proteomics. J. Insect Physiol. *57*, 179–190. https://doi.org/10.1016/j.jinsphys.2010.11.001

Cilia, M., Bereman, M., Fish, T., MacCoss, M.J., and Gray, S. (2012a). Homopteran vector biomarkers for efficient circulative plant virus transmission are conserved in multiple aphid species and the whitefly *Bemisia tabaci*. J. Integr. Agr. *11*, 249–262. https://doi.org/10.1016/S2095-3119(12)60009-4

Cilia, M., Peter, K.A., Bereman, M.S., Howe, K., Fish, T., Smith, D., Gildow, F., MacCoss, M.J., Thannhauser, T.W., and Gray, S.M. (2012b). Discovery and targeted LC-MS/MS of purified polerovirus reveals differences in the virus-host interactome associated with altered aphid transmission. PLOS ONE *7*, e48177. https://doi.org/10.1371/journal.pone.0048177

Claudel, P., Chesnais, Q., Fouche, Q., Krieger, C., Halter, D., Bogaert, F., Meyer, S., Boissinot, S., Hugueney, P., Ziegler-Graff, V., *et al.* (2018). The aphid-transmitted *Turnip yellows virus* differentially affects volatiles emission and subsequent vector behavior in two Brassicaceae plants. Int. J. Mol. Sci. *19*. https://doi.org/10.3390/ijms19082316

Consortium, I.A.G. (2010). Genome sequence of the pea aphid *Acyrthosiphon pisum*. PLOS Biol. *8*, e1000313. https://doi.org/10.1371/journal.pbio.1000313

Czosnek, H., Hariton-Shalev, A., Sobol, I., Gorovits, R., and Ghanim, M. (2017). The incredible journey of begomoviruses in their whitefly vector. Viruses *9*, E273.

DeBlasio, S.L., Chavez, J.D., Alexander, M.M., Ramsey, J., Eng, J.K., Mahoney, J., Gray, S.M., Bruce, J.E., and Cilia, M. (2015a). Visualization of host-polerovirus interaction topologies using protein interaction reporter technology. J. Virol. *90*, 1973–1987. https://doi.org/10.1128/JVI.01706-15

DeBlasio, S.L., Johnson, R., Mahoney, J., Karasev, A., Gray, S.M., MacCoss, M.J., and Cilia, M. (2015b). Insights into the polerovirus-plant interactome revealed by coimmunoprecipitation and mass spectrometry. Mol. Plant Microbe Interact. *28*, 467–481. https://doi.org/10.1094/MPMI-11-14-0363-R

DeBlasio, S.L., Johnson, R., Sweeney, M.M., Karasev, A., Gray, S.M., MacCoss, M.J., and Cilia, M. (2015c). *Potato leafroll virus* structural proteins manipulate overlapping, yet distinct protein interaction networks during infection. Proteomics *15*, 2098–2112. https://doi.org/10.1002/pmic.201400594

DeBlasio, S.L., Bereman, M.S., Mahoney, J., Thannhauser, T.W., Gray, S.M., MacCoss, M.J., and Cilia Heck, M. (2017). Evaluation of a bead-free coimmunoprecipitation technique for identification of virus-host protein interactions using high-resolution mass spectrometry. J. Biomol. Tech. *28*, 111–121. https://doi.org/10.7171/jbt.17-2803-002

DeBlasio, S.L., Rebelo, A.R., Parks, K., Gray, S.M., and Heck, M.C. (2018). Disruption of chloroplast function through downregulation of phytoene desaturase enhances the systemic accumulation of an aphid-borne, phloem-restricted virus. Mol. Plant Microbe Interact. *31*, 1095–1110. https://doi.org/10.1094/MPMI-03-18-0057-R

Di Carli, M., Benvenuto, E., and Donini, M. (2012). Recent insights into plant-virus interactions through proteomic analysis. J. Proteome Res. *11*, 4765–4780. https://doi.org/10.1021/pr300494e

Dombrovsky, A., Gollop, N., Chen, S., Chejanovsky, N., and Raccah, B. (2007). *In vitro* association between the helper component-proteinase of *Zucchini yellow mosaic virus* and cuticle proteins of *Myzus persicae*. J. Gen. Virol. *88*, 1602–1610. https://doi.org/10.1099/vir.0.82769-0

Donaldson, J.R., and Gratton, C. (2007). Antagonistic effects of soybean viruses on soybean aphid performance. Environ. Entomol. *36*, 918–925.

Drucker, M., and Then, C. (2015). Transmission activation in non-circulative virus transmission: a general concept? Curr. Opin. Virol. *15*, 63–68. https://doi.org/10.1016/j.coviro.2015.08.006

Drucker, M., Froissart, R., Hébrard, E., Uzest, M., Ravallec, M., Espérandieu, P., Mani, J.C., Pugnière, M., Roquet, F., Fereres, A., *et al*. (2002). Intracellular distribution of viral gene products regulates a complex mechanism of *Cauliflower mosaic virus* acquisition by its aphid vector. Proc. Natl. Acad. Sci. U.S.A. *99*, 2422–2427. https://doi.org/10.1073/pnas.042587799

Durvasula, R.V., Sundaram, R.K., Cordon-Rosales, C., Pennington, P., and Beard, B. (2003). *Rhodnius prolixus* and its symbiont, *Rhodococcus rhodnii*: a model for paratransgenic control of disease transmission. In Insect Symbiosis, Miller, T.A., ed. (CRC Press, Boca Raton, FL, USA), pp. 83–95.

Eigenbrode, S.D., Ding, H., Shiel, P., and Berger, P.H. (2002). Volatiles from potato plants infected with *Potato leafroll virus* attract and arrest the virus vector, *Myzus persicae* (Homoptera: Aphididae). Proc. Biol. Sci. *269*, 455–460. https://doi.org/10.1098/rspb.2001.1909

Espinoza, A.M., Medina, V., Hull, R., and Markham, P.G. (1991). *Cauliflower mosaic virus* gene II product forms distinct inclusion bodies in infected plant cells. Virology *185*, 337–344.

Fédière, G. (2000). Epidemiology and pathology of *Densovirinae*. Contrib. Microbiol. *4*, 1–11.

Fiebig, M., Poehling, H.M., and Borgemeister, C. (2004). *Barley yellow dwarf virus*, wheat, and *Sitobion avenae*: a case of trilateral interactions. Entomol. Exp. Appl. *110*, 11–21.

Franz, A.W., van der Wilk, F., Verbeek, M., Dullemans, A.M., and van den Heuvel, J.F. (1999). *Faba bean necrotic yellows virus* (genus *Nanovirus*) requires a helper factor for its aphid transmission. Virology *262*, 210–219. https://doi.org/10.1006/viro.1999.9904

Gaedigk, K., Adam, G., and Mundry, K.-W. (1986). The spike protein of *Potato yellow dwarf virus* and its functional role in the infection of insect vector cells. J. Gen. Virol. *67*, 2763–2773. https://doi.org/10.1099/0022-1317-67-12-2763

Garret, A., Kerlan, C., and Thomas, D. (1993). The intestine is a site of passage for *Potato leafroll virus* from the gut lumen into the haemocoel in the aphid vector, *Myzus persicae* Sulz. Arch. Virol. *131*, 377–392. https://doi.org/10.1007/bf01378639

Garret, A., Kerlan, C., and Thomas, D. (1996). Ultrastructural study of acquisition and retention of *Potato leafroll* luteovirus in the alimentary canal of its aphid vector, *Myzus persicae* Sulz. Arch. Virol. *141*, 1279–1292. https://doi.org/10.1007/bf01718830

Gallet, R., Fabre, F., Thébaud, G., Sofonea, M.T., Sicard, A., Blanc, S., and Michalakis, Y. (2018). Small bottleneck size in a highly multipartite virus during a complete infection cycle. J. Virol. *92*, e00139–00118. https://doi.org/10.1128/JVI.00139

Garry, C.E., and Garry, R.F. (2004). Proteomics computational analyses suggest that the carboxyl terminal glycoproteins of bunyaviruses are class II viral fusion protein (beta-penetrenes). Theor. Biol. Med. Model. *1*, 10. https://doi.org/10.1186/1742-4682-1-10

Geng, L., Qian, L.X., Shao, R.X., Liu, Y.Q., Liu, S.S., and Wang, X.W. (2018). Transcriptome profiling of whitefly guts in response to *Tomato yellow leaf curl virus* infection. Virol. J. *15*, 14. https://doi.org/10.1186/s12985-018-0926-6

Ghanim, M., and Medina, V. (2007). Localization of *Tomato yellow leaf curl virus* in its whitefly vector *Bemisia tabaci*. In Tomato Yellow Leaf Curl Virus Disease: Management, Molecular Biology, Breeding for Resistance, H. Czosnek, ed. (Springer Netherlands, Dordrecht), pp. 171–183.

Ghanim, M., Fattah-Hosseini, S., Levy, A., and Cilia, M. (2016). Morphological abnormalities and cell death in the Asian citrus psyllid (*Diaphorina citri*) midgut associated with Candidatus *Liberibacter asiaticus*. Sci. Rep. *6*, 33418. https://doi.org/10.1038/srep33418

Gildow, F.E. (1980). Increased production of alatae by aphids reared on oats infected with *Barley yellow dwarf virus*. Ann. Entomol. Soc. Am. *73*, 343347.

Gildow, F.E. (1983). Influence of *Barley yellow dwarf virus*-infected oats and barley on morphology of aphid vectors. Phytopathology *73*, 1196–1199. https://doi.org/10.1094/Phyto-73-1196

Gildow, F.E. (1987). Virus-membrane interactions involved in circulative transmission of luteoviruses by aphids. In Current Topics in Vector Research, K.F. Harris, ed. (Springer New York, New York, NY), pp. 93–120.

Gildow, F.E. (1993). Evidence for receptor-mediated endocytosis regulating luteovirus acquisition by aphids. Phytopathology *83*, 270–277. https://doi.org/10.1094/Phyto-83-270

Götz, M., Popovski, S., Kollenberg, M., Gorovits, R., Brown, J.K., Cicero, J.M., Czosnek, H., Winter, S., and Ghanim, M. (2012). Implication of *Bemisia tabaci* heat shock protein 70 in begomovirus-whitefly interactions. J. Virol. *86*, 13241–13252. https://doi.org/10.1128/JVI.00880-12

Govier, D.A., and Kassanis, B. (1974). A virus-induced component of plant sap needed when aphids acquire

Potato virus Y from purified preparations. Virology *61*, 420–426. https://doi.org/10.1016/0042-6822(74)90278-5

Gray, S.M., Smith, D.M., Barbierri, L., and Burd, J. (2002). Virus transmission phenotype is correlated with host adaptation among genetically diverse populations of the aphid *Schizaphis graminum*. Phytopathology *92*, 970–975. https://doi.org/10.1094/PHYTO.2002.92.9.970

Gray, S.M., Caillaud, M.C., Burrows, M., and Smith, D.M. (2007). Transmission of two viruses that cause Barley Yellow Dwarf is controlled by different loci in the aphid, *Schizaphis graminum*. J. Insect Sci. *7*, 1–15. https://doi.org/10.1673/031.007.2501

Gray, S.M., Cilia, M., and Ghanim, M. (2014). Circulative, 'nonpropagative' virus transmission: an orchestra of virus-, insect-, and plant-derived instruments. Adv. Virus Res. *89*, 141–199. https://doi.org/10.1016/B978-0-12-800172-1.00004-5

Grigoras, I., Vetten, H.J., Commandeur, U., Ziebell, H., Gronenborn, B., and Timchenko, T. (2018). Nanovirus DNA-N encodes a protein mandatory for aphid transmission. Virology *522*, 281–291.

Groen, S.C., Jiang, S., Murphy, A.M., Cunniffe, N.J., Westwood, J.H., Davey, M.P., Bruce, T.J., Caulfield, J.C., Furzer, O.J., Reed, A., *et al.* (2016). Virus infection of plants alters pollinator preference: A payback for susceptible hosts? PLOS Pathog. *12*, e1005790. https://doi.org/10.1371/journal.ppat.1005790

Harrison, B.D., and Robinson, D.J. (1988). Molecular variation in vector-borne plant viruses: epidemiological significance. Philos. Trans. R. Soc. Lond., B, Biol. Sci. *321*, 447–462. https://doi.org/10.1098/rstb.1988.0102

He, D.C., Zhan, J., Cheng, Z.B., and Xie, L.H. (2016). Viruliferous rate of small brown planthopper is a good indicator of rice stripe disease epidemics. Sci. Rep. *6*, 21376. https://doi.org/10.1038/srep21376

He, W.B., Li, J., and Liu, S.S. (2015). Differential profiles of direct and indirect modification of vector feeding behaviour by a plant virus. Sci. Rep. *5*, 7682. https://doi.org/10.1038/srep07682

Heck, M. (2018). Insect transmission of plant pathogens: a systems biology perspective. mSystems *3*, e00168–17. https://doi.org/10.1128/mSystems.00168-17

Heck, M., and Brault, V. (2018). Targeted disruption of aphid transmission: a vision for the management of crop diseases caused by *Luteoviridae* members. Curr. Opin. Virol. *33*, 24–32.

Heil, M. (2016). Host manipulation by parasites: cases, patterns, and remaining doubts. Front. Ecol. Evol. *4*. https://doi.org/10.3389/fevo.2016.00080

Hodge, S., and Powell, G. (2008). Do plant viruses facilitate their aphid vectors by inducing symptoms that alter behavior and performance? Environ. Entomol. *37*, 1573–1581.

Höfer, P., Bedford, I.D., Markham, P.G., Jeske, H., and Frischmuth, T. (1997). Coat protein gene replacement results in whitefly transmission of an insect nontransmissible geminivirus isolate. Virology *236*, 288–295.

Hoffmann, A.A., Montgomery, B.L., Popovici, J., Iturbe-Ormaetxe, I., Johnson, P.H., Muzzi, F., Greenfield, M., Durkan, M., Leong, Y.S., Dong, Y., *et al.* (2011). Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. Nature *476*, 454–457. https://doi.org/10.1038/nature10356

Höhnle, M., Höfer, P., Bedford, I.D., Briddon, R.W., Markham, P.G., and Frischmuth, T. (2001). Exchange of three amino acids in the coat protein results in efficient whitefly transmission of a nontransmissible *Abutilon mosaic virus* isolate. Virology *290*, 164–171. https://doi.org/10.1006/viro.2001.1140

Holmes, J.C., and Bethel, W.M. (1972). Modification of intermediate host behaviour by parasites. In Behavioural Aspects of Parasite Transmission, E.U. Canning, and C.A. Wright, eds. (Academic Press, London and New York), pp. 123–149.

Holopainen, J.K., Semiz, G., and Blande, J.D. (2009). Life-history strategies affect aphid preference for yellowing leaves. Biol. Lett. *5*, 603–605. https://doi.org/10.1098/rsbl.2009.0372

Huang, H.J., Bao, Y.Y., Lao, S.H., Huang, X.H., Ye, Y.Z., Wu, J.X., Xu, H.J., Zhou, X.P., and Zhang, C.X. (2015). *Rice ragged stunt virus*-induced apoptosis affects virus transmission from its insect vector, the brown planthopper to the rice plant. Sci. Rep. *5*, 11413. https://doi.org/10.1038/srep11413

Huang, Y.W., Geng, Y.F., Ying, X.B., Chen, X.Y., and Fang, R.X. (2005). Identification of a movement protein of *Rice yellow stunt* rhabdovirus. J. Virol. *79*, 2108–2114. https://doi.org/10.1128/JVI.79.4.2108-2114.2005

Huo, Y., Liu, W., Zhang, F., Chen, X., Li, L., Liu, Q., Zhou, Y., Wei, T., Fang, R., and Wang, X. (2014). Transovarial transmission of a plant virus is mediated by vitellogenin of its insect vector. PLOS Pathog. *10*, e1003949. https://doi.org/10.1371/journal.ppat.1003949

Ingwell, L.L., Eigenbrode, S.D., and Bosque-Pérez, N.A. (2012). Plant viruses alter insect behavior to enhance their spread. Sci. Rep. *2*, 578. https://doi.org/10.1038/srep00578

Ivanov, K.I., Eskelin, K., Bašić, M., De, S., Lõhmus, A., Varjosalo, M., and Mäkinen, K. (2016). Molecular insights into the function of the viral RNA silencing suppressor HCPro. Plant J. *85*, 30–45. https://doi.org/10.1111/tpj.13088

Jackson, A.O., Dietzgen, R.G., Goodin, M.M., Bragg, J.N., and Deng, M. (2005). Biology of plant rhabdoviruses. Annu. Rev. Phytopathol. *43*, 623–660. https://doi.org/10.1146/annurev.phyto.43.011205.141136

Jacquemond, M. (2012). *Cucumber mosaic virus*. Adv. Virus Res. *84*. https://doi.org/10.1016/S0065-3527(08)60039-1

Jensen, S.G. (1972). Metabolism and carbohydrate composition in *Barley yellow dwarf virus*-infected wheat. Phytopathology *62*, 587–592.

Jiménez-Martíneza, E.S., Bosque-Pérez, S.A., Berger, P.H., Zemetrad, R.S., Ding, H., and Eigenbrode, S.D. (2004). Volatile cues influence the response of *Rhopalosiphum padi* (Homoptera: Aphididae) to *Barley yellow dwarf virus*–infected transgenic and untransformed wheat. Environ. Entomol. *33*, 1207–1216. https://doi.org/10.1603/0046-225X-33.5.1207

Kanakala, S., and Ghanim, M. (2016). Implication of the whitefly *Bemisia tabaci* Cyclophilin B protein in the transmission of *Tomato yellow leaf curl virus*. Front. Plant Sci. *7*, 1702. https://doi.org/10.3389/fpls.2016.01702

Kaplan, I.B., Lee, L., Ripoll, D.R., Palukaitis, P., Gildow, F., and Gray, S.M. (2007). Point mutations in the *Potato leafroll virus* major capsid protein alter virion stability and aphid transmission. J. Gen. Virol. *88*, 1821–1830. https://doi.org/10.1099/vir.0.82837-0

Kassanis, B., and Govier, D.A. (1971). New evidence on the mechanism of aphid transmission of *Potato C* and *Potato aucuba mosaic* viruses. J. Gen. Virol. *10*, 99–101. https://doi.org/10.1099/0022-1317-10-1-99

Kaur, N., Hasegawa, D.K., Ling, K.S., and Wintermantel, W.M. (2016). Application of genomics for understanding plant virus-insect vector interactions and insect vector control. Phytopathology *106*, 1213–1222. https://doi.org/10.1094/PHYTO-02-16-0111-FI

Kersten, B., Ghirardo, A., Schnitzler, J.P., Kanawati, B., Schmitt-Kopplin, P., Fladung, M., and Schroeder, H. (2013). Integrated transcriptomics and metabolomics decipher differences in the resistance of pedunculate oak to the herbivore *Tortrix viridana* L. BMC Genomics *14*, 737. https://doi.org/10.1186/1471-2164-14-737

Khelifa, M., Journou, S., Krishnan, K., Gargani, D., Esperandieu, P., Blanc, S., and Drucker, M. (2007). Electron-lucent inclusion bodies are structures specialized for aphid transmission of *Cauliflower mosaic virus*. J. Gen. Virol. *88*, 2872–2880. https://doi.org/10.1099/vir.0.83009-0

Kikkert, M., Meurs, C., van de Wetering, F., Dorfmüller, S., Peters, D., Kormelink, R., and Goldbach, R. (1998). Binding of *Tomato spotted wilt virus* to a 94-kDa thrips protein. Phytopathology *88*, 63–69. https://doi.org/10.1094/PHYTO.1998.88.1.63

Kotzampigikis, A., Hristova, D., and Tasheva-Terzieva, E. (2010). Virus-vector relationship between *Potato leafroll virus* PLRV and *Myzus persicae* Sulzer. Bulg. J. Agri. Sci. *16*, 412–421.

Krenz, B., Schießl, I., Greiner, E., and Krapp, S. (2017). Analyses of *Pea necrotic yellow dwarf virus*-encoded proteins. Virus Genes *53*, 454–463. https://doi.org/10.1007/s11262-017-1439-x

Kritzman, A., Gera, A., Raccah, B., van Lent, J.W., and Peters, D. (2002). The route of *Tomato spotted wilt virus* inside the thrips body in relation to transmission efficiency. Arch. Virol. *147*, 2143–2156. https://doi.org/10.1007/s00705-002-0871-x

Kruse, A., Fattah-Hosseini, S., Saha, S., Johnson, R., Warwick, E., Sturgeon, K., Mueller, L., MacCoss, M.J., Shatters, R.G., and Cilia Heck, M. (2017). Combining 'omics and microscopy to visualize interactions between the Asian citrus psyllid vector and the Huanglongbing pathogen Candidatus *Liberibacter asiaticus* in the insect gut. PLOS ONE *12*, e0179531. https://doi.org/10.1371/journal.pone.0179531

Kruse, A., Ramsey, J.S., Johnson, R., Hall, D.G., MacCoss, M.J., and Heck, M. (2018). Candidatus *Liberibacter asiaticus* minimally alters expression of immunity and metabolism proteins in hemolymph of *Diaphorina citri*, the insect vector of Huanglongbing. J. Proteome Res. *17*, 2995–3011. https://doi.org/10.1021/acs.jproteome.8b00183

Kukurba, K.R., and Montgomery, S.B. (2015). RNA sequencing and analysis. Cold Spring Harb. Protoc. *2015*, 951–969. https://doi.org/10.1101/pdb.top084970

Lan, H., Chen, H., Liu, Y., Jiang, C., Mao, Q., Jia, D., Chen, Q., and Wei, T. (2016a). Small interfering RNA pathway modulates initial viral infection in midgut epithelium of insect after ingestion of virus. J. Virol. *90*, 917–929. https://doi.org/10.1128/JVI.01835-15

Lan, H., Wang, H., Chen, Q., Chen, H., Jia, D., Mao, Q., and Wei, T. (2016b). Small interfering RNA pathway modulates persistent infection of a plant virus in its insect vector. Sci. Rep. *6*, 20699. https://doi.org/10.1038/srep20699

Lee, L., Kaplan, I.B., Ripoll, D.R., Liang, D., Palukaitis, P., and Gray, S.M. (2005). A surface loop of the *Potato leafroll virus* coat protein is involved in virion assembly, systemic movement, and aphid transmission. J. Virol. *79*, 1207–1214.

Leh, V., Jacquot, E., Geldreich, A., Hermann, T., Leclerc, D., Cerutti, M., Yot, P., Keller, M., and Blanc, S. (1999). Aphid transmission of *Cauliflower mosaic virus* requires the viral PIII protein. EMBO J. *18*, 7077–7085. https://doi.org/10.1093/emboj/18.24.7077

Leh, V., Jacquot, E., Geldreich, A., Haas, M., Blanc, S., Keller, M., and Yot, P. (2001). Interaction between the open reading frame III product and the coat protein is required for transmission of *Cauliflower mosaic virus* by aphids. J. Virol. *75*, 100–106. https://doi.org/10.1128/JVI.75.1.100-106.2001

Li, C., Cox-Foster, D., Gray, S.M., and Gildow, F. (2001). Vector specificity of *Barley yellow dwarf virus* (BYDV) transmission: identification of potential cellular receptors binding BYDV-MAV in the aphid, *Sitobion avenae*. Virology *286*, 125–133. https://doi.org/10.1006/viro.2001.0929

Li, S., Xiong, R., Wang, X., and Zhou, Y. (2011). Five proteins of *Laodelphax striatellus* are potentially involved in the interactions between *Rice stripe virus* and vector. PLOS ONE *6*, e26585. https://doi.org/10.1371/journal.pone.0026585

Li, S., Wang, S., Wang, X., Li, X., Zi, J., Ge, S., Cheng, Z., Zhou, T., Ji, Y., Deng, J., *et al.* (2015). *Rice stripe virus* affects the viability of its vector offspring by changing developmental gene expression in embryos. Sci. Rep. *5*, 7883. https://doi.org/10.1038/srep07883

Li, S., Li, X., and Zhou, Y. (2018). Ribosomal protein L18 is an essential factor that promote *Rice stripe virus* accumulation in small brown planthopper. Virus Res. *247*, 15–20.

Li, S., Zhou, C., and Zhou, Y. (2019). Olfactory co-receptor Orco stimulated by *Rice stripe virus* is essential for host seeking behavior in small brown planthopper. Pest Manag. Sci. *75*, 187–194. https://doi.org/10.1002/ps.5086

Liang, Y., and Gao, X.W. (2017). The cuticle protein gene MPCP4 of *Myzus persicae* (Homoptera: Aphididae) plays a critical role in *Cucumber mosaic virus* acquisition. J. Econ. Entomol. *110*, 848–853. https://doi.org/10.1093/jee/tox025

Lightle, D., and Lee, J. (2014). Raspberry viruses affect the behavior and performance of *Amphorophora agathonica* in single and mixed infections. Entomol. Exp. Appl. *151*, 57–64. https://doi.org/10.1111/eea.12170

Linz, L.B., Liu, S., Chougule, N.P., and Bonning, B.C. (2015). *In vitro* evidence supports membrane Alanyl Aminopeptidase N as a receptor for a plant virus in the

pea aphid vector. J. Virol. *89*, 11203–11212. https://doi.org/10.1128/JVI.01479-15

Liu, B., Preisser, E.L., Chu, D., Pan, H., Xie, W., Wang, S., Wu, Q., Zhou, X., and Zhang, Y. (2013). Multiple forms of vector manipulation by a plant-infecting virus: *Bemisia tabaci* and *Tomato yellow leaf curl virus*. J. Virol. *87*, 4929–4937. https://doi.org/10.1128/JVI.03571-12

Liu, S., Briddon, R.W., Bedford, I.D., Pinner, M.S., and Markham, P.G. (1999). Identification of genes directly and indirectly involved in the insect transmission of *African cassava mosaic* geminivirus by *Bemisia tabaci*. Virus Genes *18*, 5–11. https://doi.org/10.1023/A:1008017017020

Liu, S., He, X., Park, G., Josefsson, C., and Perry, K.L. (2002). A conserved capsid protein surface domain of *Cucumber mosaic virus* is essential for efficient aphid vector transmission. J. Virol. *76*, 9756–9762. https://doi.org/10.1128/JVI.76.19.9756-9762.2002

Liu, S., Sivakumar, S., Sparks, W.O., Miller, W.A., and Bonning, B.C. (2010). A peptide that binds the pea aphid gut impedes entry of *Pea enation mosaic virus* into the aphid hemocoel. Virology *401*, 107–116. https://doi.org/10.1016/j.virol.2010.02.009

Liu, W., Gray, S., Huo, Y., Li, L., Wei, T., and Wang, X. (2015). Proteomic analysis of interaction between a plant virus and its vector insect reveals new functions of hemipteran cuticular protein. Mol. Cell Proteomics *14*, 2229–2242. https://doi.org/10.1074/mcp.M114.046763

Liu, Y., Jia, D., Chen, H., Chen, Q., Xie, L., Wu, Z., and Wei, T. (2011). The P7-1 protein of *Southern rice black-streaked dwarf virus*, a fijivirus, induces the formation of tubular structures in insect cells. Arch. Virol. *156*, 1729–1736. https://doi.org/10.1007/s00705-011-1041-9

Luan, J.B., Li, J.M., Varela, N., Wang, Y.L., Li, F.F., Bao, Y.Y., Zhang, C.X., Liu, S.S., and Wang, X.W. (2011). Global analysis of the transcriptional response of whitefly to *Tomato yellow leaf curl China virus* reveals the relationship of coevolved adaptations. J. Virol. *85*, 3330–3340. https://doi.org/10.1128/JVI.02507-10

Maag, D., Erb, M., and Glauser, G. (2015). Metabolomics in plant-herbivore interactions: challenges and applications. Entomol. Exp. Appl. *157*, 18–29. https://doi.org/10.1111/eea.12336

Macias, W., and Mink, G.I. (1969). Preference of green peach aphid for virus-infected sugarbeet leaves. J. Econ. Entomol. *62*, 28–29. https://doi.org/10.1093/jee/62.1.28

Maluta, N.K., Garzo, E., Moreno, A., Lopes, J.R., and Fereres, A. (2014). *Tomato yellow leaf curl virus* benefits population growth of the Q Biotype of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). Neotrop. Entomol. *43*, 385–392. https://doi.org/10.1007/s13744-014-0223-z

Mann, M., Fattah-Hosseini, S., Ammar, E.D., Stange, R., Warrick, E., Sturgeon, K., Shatters, R., and Heck, M. (2018). *Diaphorina citri* nymphs are resistant to morphological changes induced by 'Candidatus *Liberibacter asiaticus*' in midgut epithelial cells. Infect. Immun. *86*, e00889–17.

Mann, R.S., Ali, J.G., Hermann, S.L., Tiwari, S., Pelz-Stelinski, K.S., Alborn, H.T., and Stelinski, L.L. (2012). Induced release of a plant-defense volatile 'deceptively' attracts insect vectors to plants infected with a bacterial pathogen. PLOS Pathog. *8*, e1002610. https://doi.org/10.1371/journal.ppat.1002610

Mar, T., Liu, W., and Wang, X. (2014). Proteomic analysis of interaction between P7-1 of *Southern rice black-streaked dwarf virus* and the insect vector reveals diverse insect proteins involved in successful transmission. J. Proteomics *102*, 83–97. https://doi.org/10.1016/j.jprot.2014.03.004

Maris, P.C., Joosten, N.N., Goldbach, R.W., and Peters, D. (2004). *Tomato spotted wilt virus* infection improves host suitability for its vector *Frankliniella occidentalis*. Phytopathology *94*, 706–711. https://doi.org/10.1094/PHYTO.2004.94.7.706

Martini, X., Pelz-Stelinski, K.S., and Stelinski, L.L. (2014). Plant pathogen-induced volatiles attract parasitoids to increase parasitism of an insect vector. Front. Ecol. Evol. *2*. https://doi.org/10.3389/fevo.2014.00008

Martinière, A., Bak, A., Macia, J.L., Lautredou, N., Gargani, D., Doumayrou, J., Garzo, E., Moreno, A., Fereres, A., Blanc, S., *et al.* (2013). A virus responds instantly to the presence of the vector on the host and forms transmission morphs. Elife *2*, e00183. https://doi.org/10.7554/eLife.00183

Mathers, T.C., Chen, Y., Kaithakottil, G., Legeai, F., Mugford, S.T., Baa-Puyoulet, P., Bretaudeau, A., Clavijo, B., Colella, S., Collin, O., *et al.* (2017). Rapid transcriptional plasticity of duplicated gene clusters enables a clonally reproducing aphid to colonise diverse plant species. Genome Biol. *18*, 27. https://doi.org/10.1186/s13059-016-1145-3

Mauck, K.E., De Moraes, C.M., and Mescher, M.C. (2010a). Deceptive chemical signals induced by a plant virus attract insect vectors to inferior hosts. Proc. Natl. Acad. Sci. U.S.A. *107*, 3600–3605. https://doi.org/10.1073/pnas.0907191107

Mauck, K.E., De Moraes, C.M., and Mescher, M.C. (2010b). Effects of *Cucumber mosaic virus* infection on vector and non-vector herbivores of squash. Commun. Integr. Biol. *3*, 579–582. https://doi.org/10.4161/cib.3.6.13094

Mauck, K.E., Bosque-Perez, N.A., Eigenbrode, S.D., De Moraes, C.M., and Mescher, M.C. (2012). Transmission mechanisms shape pathogen effects on host-vector interactions: evidence from plant viruses. Funct. Ecol. *26*, 1162–1175. https://doi.org/10.1111/j.1365-2435.2012.02026.x

Mauck, K.E., De Moraes, C.M., and Mescher, M.C. (2014). Biochemical and physiological mechanisms underlying effects of *Cucumber mosaic virus* on host-plant traits that mediate transmission by aphid vectors. Plant Cell Environ. *37*, 1427–1439. https://doi.org/10.1111/pce.12249

McMenemy, L.S., Hartley, S.E., MacFarlane, S.A., Karley, A.J., Shepherd, T., and Johnson, S.N. (2012). Raspberry viruses manipulate the behaviour of their insect vectors. Entomol. Exp. Appl. *144*, 56–68. https://doi.org/10.1111/j.1570-7458.2012.01248.x

Medina-Ortega, K.J., Bosque-Pérez, N.A., Ngumbi, E., Jiménez-Martínez, E.S., and Eigenbrode, S.D. (2009). *Rhopalosiphum padi* (Hemiptera: Aphididae) responses to volatile cues from *Barley yellow dwarf virus*-infected wheat. Environ. Entomol. *38*, 836–845. https://doi.org/10.1603/022.038.0337

Mittler, T.E. (1967). Effect of amino acid and sugar concentrations on the food uptake of the aphid *Myzus persicae*. Entomol. Exp. Appl. *10*, 39–51. https://doi.org/10.1111/j.1570-7458.1967.tb00043.x

Moritz, G., Kumm, S., and Mound, L. (2004). Tospovirus transmission depends on thrips ontogeny. Virus Res. *100*, 143–149. https://doi.org/10.1016/j.virusres.2003.12.022

Morkunas, I., Mai, V.C., and Gabryś, B. (2011). Phytohormonal signaling in plant responses to aphid feeding. Acta Physiol. Plant. *33*, 2057–2073. https://doi.org/10.1007/s11738-011-0751-7

Mossop, D.W., and Francki, R.I. (1977). Association of RNA 3 with aphid transmission of *Cucumber mosaic virus*. Virology *81*, 177–181. https://doi.org/10.1016/0042-6822(77)90071-X

Mulot, M., Monsion, B., Boissinot, S., Rastegar, M., Meyer, S., Bochet, N., and Brault, V. (2018). Transmission of *Turnip yellows virus* by *Myzus persicae* is reduced by feeding aphids on double-stranded RNA targeting the ephrin receptor protein. Front. Microbiol. *9*, 457. https://doi.org/10.3389/fmicb.2018.00457

Nagata, T., Inoue-Nagata, A.K., Smid, H.M., Goldbach, R., and Peters, D. (1999). Tissue tropism related to vector competence of *Frankliniella occidentalis* for *Tomato spotted wilt* tospovirus. J. Gen. Virol. *80*, 507–515. https://doi.org/10.1099/0022-1317-80-2-507

Nasu, S. (1963). Studies on some leafhoppers and planthoppers which transmit virus diseases of rice plant in Japan. Bull. Kyushu Agric. Exp. Stn. *8*, 153–349.

Ng, J.C., Liu, S., and Perry, K.L. (2000). *Cucumber mosaic virus* mutants with altered physical properties and defective in aphid vector transmission. Virology *276*, 395–403. https://doi.org/10.1006/viro.2000.0569

Ng, J.C., Josefsson, C., Clark, A.J., Franz, A.W., and Perry, K.L. (2005). Virion stability and aphid vector transmissibility of *Cucumber mosaic virus* mutants. Virology *332*, 397–405.

Ngumbi, E., Eigenbrode, S.D., Bosque-Pérez, N.A., Ding, H., and Rodriguez, A. (2007). *Myzus persicae* is arrested more by blends than by individual compounds elevated in headspace of PLRV-infected potato. J. Chem. Ecol. *33*, 1733–1747. https://doi.org/10.1007/s10886-007-9340-z

Noris, E., Vaira, A.M., Caciagli, P., Masenga, V., Gronenborn, B., and Accotto, G.P. (1998). Amino acids in the capsid protein of *Tomato yellow leaf curl virus* that are crucial for systemic infection, particle formation, and insect transmission. J. Virol. *72*, 10050–10057.

Nouri, S., Matsumura, E.E., Kuo, Y.W., and Falk, B.W. (2018). Insect-specific viruses: from discovery to potential translational applications. Curr. Opin. Virol. *33*, 33–41.

Omura, T., Yan, J., Zhong, B., Wada, M., Zhu, Y., Tomaru, M., Maruyama, W., Kikuchi, A., Watanabe, Y., Kimura, I., *et al.* (1998). The P2 protein of *Rice dwarf* phytoreovirus is required for adsorption of the virus to cells of the insect vector. J. Virol. *72*, 9370–9373.

Oppenheim, S.J., Baker, R.H., Simon, S., and DeSalle, R. (2015). We can't all be supermodels: the value of comparative transcriptomics to the study of non-model insects. Insect Mol. Biol. *24*, 139–154. https://doi.org/10.1111/imb.12154

Pakkianathan, B.C., Kontsedalov, S., Lebedev, G., Mahadav, A., Zeidan, M., Czosnek, H., and Ghanim, M. (2015). Replication of *Tomato yellow leaf curl virus* in its whitefly vector, *Bemisia tabaci*. J. Virol. *89*, 9791–9803. https://doi.org/10.1128/JVI.00779-15

Pallas, V., and Garcia, J.A. (2011). How do plant viruses induce disease? Interactions and interference with host components. J. Gen. Virol. *92*, 2691–2705. https://doi.org/10.1099/vir.0.034603-0

Papura, D., Jacquot, E., Dedryver, C.A., Luche, S., Riault, G., Bossis, M., and Rabilloud, T. (2002). Two-dimensional electrophoresis of proteins discriminates aphid clones of *Sitobion avenae* differing in BYDV-PAV transmission. Arch. Virol. *147*, 1881–1898. https://doi.org/10.1007/s00705-002-0859-6

Peiffer, M.L., Gildow, F.E., and Gray, S.M. (1997). Two distinct mechanisms regulate luteovirus transmission efficiency and specificity at the aphid salivary gland. J. Gen. Virol. *78*, 495–503. https://doi.org/10.1099/0022-1317-78-3-495

Pelz-Stelinski, K.S., and Killiny, N. (2016). Better together: association with '*Candidatus Liberibacter* Asiaticus' increases the reproductive fitness of its insect vector, *Diaphorina citri* (Hemiptera: Liviidae). Ann. Entomol. Soc. Am. *109*, 371–376. https://doi.org/10.1093/aesa/saw007

Peng, Y.H., Kadoury, D., Gal-On, A., Huet, H., Wang, Y., and Raccah, B. (1998). Mutations in the HC-Pro gene of *Zucchini yellow mosaic* potyvirus: effects on aphid transmission and binding to purified virions. J. Gen. Virol. *79*, 897–904. https://doi.org/10.1099/0022-1317-79-4-897

Perry, K.L., Zhang, L., Shintaku, M.H., and Palukaitis, P. (1994). Mapping determinants in *Cucumber mosaic virus* for transmission by *Aphis gossypii*. Virology *205*, 591–595. https://doi.org/10.1006/viro.1994.1686

Perry, K.L., Zhang, L., and Palukaitis, P. (1998). Amino acid changes in the coat protein of *Cucumber mosaic virus* differentially affect transmission by the aphids *Myzus persicae* and *Aphis gossypii*. Virology *242*, 204–210. https://doi.org/10.1006/viro.1998.8991

Peter, K.A., Liang, D., Palukaitis, P., and Gray, S.M. (2008). Small deletions in the *Potato leafroll virus* readthrough protein affect particle morphology, aphid transmission, virus movement and accumulation. J. Gen. Virol. *89*, 2037–2045. https://doi.org/10.1099/vir.0.83625-0

Peter, K.A., Gildow, F., Palukaitis, P., and Gray, S.M. (2009). The C terminus of the polerovirus p5 readthrough domain limits virus infection to the phloem. J. Virol. *83*, 5419–5429. https://doi.org/10.1128/JVI.02312-08

Pinheiro, P., Bereman, M.S., Burd, J., Pals, M., Armstrong, S., Howe, K.J., Thannhauser, T.W., MacCoss, M.J., Gray, S.M., and Cilia, M. (2014). Evidence of the biochemical basis of host virulence in the greenbug aphid, *Schizaphis graminum* (Homoptera: Aphididae). J. Proteome Res. *13*, 2094–2108. https://doi.org/10.1021/pr4012415

Pinheiro, P.V., Kliot, A., Ghanim, M., and Cilia, M. (2015). Is there a role for symbiotic bacteria in plant virus transmission by insects? Curr. Opin. Insect Sci. *8*, 69–78. https://doi.org/10.1016/j.cois.2015.01.010

Pinheiro, P.V., Ghanim, M., Alexander, M., Rebelo, A.R., Santos, R.S., Orsburn, B.C., Gray, S., and Cilia, M. (2017). Host plants indirectly influence plant virus

transmission by altering gut cysteine protease activity of aphid vectors. Mol. Cell Proteomics 16 (Suppl. 4), S230–S243. https://doi.org/10.1074/mcp.M116.063495

Pinheiro, P.V., Wilson, J.R., Xu, Y., Zheng, Y., Rebelo, A.R., Fattah-Hosseini, S., Kruse, A., Santos Dos Silva, R., Xu, Y., Kramer, M., *et al.* (2019). Plant viruses transmitted in two different modes produce differing effects on small RNA-mediated processes in their insect vector. Phytobiomes J. [Epub ahead of print] https://doi.org/10.1094/PBIOMES-10-18-0045-R.

Pirone, T.P., and Megahed, S. (1966). Aphid transmissibility of some purified viruses and viral RNAs. Virology *30*, 631–637.

Pitino, M., Coleman, A.D., Maffei, M.E., Ridout, C.J., and Hogenhout, S.A. (2011). Silencing of aphid genes by dsRNA feeding from plants. PLOS ONE *6*, e25709. https://doi.org/10.1371/journal.pone.0025709

Plassmeyer, M.L., Soldan, S.S., Stachelek, K.M., Martín-García, J., and González-Scarano, F. (2005). California serogroup Gc (G1) glycoprotein is the principal determinant of pH-dependent cell fusion and entry. Virology *338*, 121–132.

Plassmeyer, M.L., Soldan, S.S., Stachelek, K.M., Roth, S.M., Martín-García, J., and González-Scarano, F. (2007). Mutagenesis of the *La Crosse virus* glycoprotein supports a role for Gc (1066-1087) as the fusion peptide. Virology *358*, 273–282.

Plisson, C., Uzest, M., Drucker, M., Froissart, R., Dumas, C., Conway, J., Thomas, D., Blanc, S., and Bron, P. (2005). Structure of the mature P3-virus particle complex of *Cauliflower mosaic virus* revealed by cryo-electron microscopy. J. Mol. Biol. *346*, 267–277.

Puterka, G.J., Nicholson, S.J., and Cooper, W.R. (2017). Survival and feeding rates of four aphid species (Hemiptera: Aphididae) on various sucrose concentrations in diets. J. Econ. Entomol. *110*, 1518–1524. https://doi.org/10.1093/jee/tox168

Rajabaskar, D., Wu, Y., Bosque-Pérez, N.A., and Eigenbrode, S.D. (2013). Dynamics of *Myzus persicae* arrestment by volatiles from *Potato leafroll virus*-infected potato plants during disease progression. Entomol. Exp. Appl. *148*, 172–181. https://doi.org/10.1111/eea.12087

Rajabaskar, D., Bosque-Pérez, N.A., and Eigenbrode, S.D. (2014). Preference by a virus vector for infected plants is reversed after virus acquisition. Virus Res. *186*, 32–37. https://doi.org/10.1016/j.virusres.2013.11.005

Ramsey, J.S., Wilson, A.C., de Vos, M., Sun, Q., Tamborindeguy, C., Winfield, A., Malloch, G., Smith, D.M., Fenton, B., Gray, S.M., *et al.* (2007). Genomic resources for *Myzus persicae*: EST sequencing, SNP identification, and microarray design. BMC Genomics *8*, 423.

Reinbold, C., Gildow, F.E., Herrbach, E., Ziegler-Graff, V., Goncalves, M.C., van Den Heuvel, J.F., and Brault, V. (2001). Studies on the role of the minor capsid protein in transport of *Beet western yellows virus* through *Myzus persicae*. J. Gen. Virol. *82*, 1995–2007. https://doi.org/10.1099/0022-1317-82-8-1995

Rochow, W.F. (1969). Biological properties of four isolates of *Barley yellow dwarf virus*. Phytopathology *59*, 1580–1589.

Rochow, W.F. (1970). *Barley yellow dwarf virus*: phenotypic mixing and vector specificity. Science *167*, 875–878. https://doi.org/10.1126/science.167.3919.875

Roumagnac, P., Granier, M., Bernardo, P., Deshoux, M., Ferdinand, R., Galzi, S., Fernandez, E., Julian, C., Abt, I., Filloux, D., *et al.* (2015). *Alfalfa leaf curl virus*: an aphid-transmitted geminivirus. J. Virol. *89*, 9683–9688. https://doi.org/10.1128/JVI.00453-15

Rubinstein, G., and Czosnek, H. (1997). Long-term association of *Tomato yellow leaf curl virus* with its whitefly vector *Bemisia tabaci*: effect on the insect transmission capacity, longevity and fecundity. J. Gen. Virol. *78*, 2683–2689. https://doi.org/10.1099/0022-1317-78-10-2683

Ryabov, E.V., Keane, G., Naish, N., Evered, C., and Winstanley, D. (2009). Densovirus induces winged morphs in asexual clones of the rosy apple aphid, *Dysaphis plantaginea*. Proc. Natl. Acad. Sci. U.S.A. *106*, 8465–8470. https://doi.org/10.1073/pnas.0901389106

Saha, S., Hosmani, P.S., Villalobos-Ayala, K., Miller, S., Shippy, T., Flores, M., Rosendale, A., Cordola, C., Bell, T., Mann, H., *et al.* (2017). Improved annotation of the insect vector of citrus greening disease: biocuration by a diverse genomics community. Database *2017*. https://doi.org/10.1093/database/bax032

Salvaudon, L., De Moraes, C.M., and Mescher, M.C. (2013). Outcomes of co-infection by two potyviruses: implications for the evolution of manipulative strategies. Proc. Biol. Sci. *280*, 20122959. https://doi.org/10.1098/rspb.2012.2959

Schlegel, R., and Wade, M. (1983). Neutralized *Vesicular stomatitis virus* binds to host cells by a different 'receptor'. Biochem. Biophys. Res. Commun. *114*, 774–778.

Schneweis, D.J., Whitfield, A.E., and Rotenberg, D. (2017). Thrips developmental stage-specific transcriptome response to *Tomato spotted wilt virus* during the virus infection cycle in *Frankliniella occidentalis*, the primary vector. Virology *500*, 226–237.

Shalitin, D., and Wolf, S. (2000). *Cucumber mosaic virus* infection affects sugar transport in melon plants. Plant Physiol. *123*, 597–604.

Shrestha, A., Champagne, D.E., Culbreath, A.K., Rotenberg, D., Whitfield, A.E., and Srinivasan, R. (2017). Transcriptome changes associated with *Tomato spotted wilt virus* infection in various life stages of its thrips vector, *Frankliniella fusca* (Hinds). J. Gen. Virol. *98*, 2156–2170. https://doi.org/10.1099/jgv.0.000874

Shukla, D.D., Strike, P.M., Tracy, S.L., Gough, K.H., and Ward, C.W. (1988). The N and C termini of the coat proteins of potyviruses are surface-located and the N terminus contains the major virus-specific epitopes. J. Gen. Virol. *69*, 1497–1508. https://doi.org/10.1099/0022-1317-69-7-1497

Sin, S.H., McNulty, B.C., Kennedy, G.G., and Moyer, J.W. (2005). Viral genetic determinants for thrips transmission of *Tomato spotted wilt virus*. Proc. Natl. Acad. Sci. U.S.A. *102*, 5168–5173.

Soto, M.J., Chen, L.F., Seo, Y.S., and Gilbertson, R.L. (2005). Identification of regions of the *Beet mild curly top virus* (family *Geminiviridae*) capsid protein involved in systemic infection, virion formation and leafhopper transmission. Virology *341*, 257–270.

Tamborindeguy, C., Bereman, M.S., DeBlasio, S., Igwe, D., Smith, D.M., White, F., MacCoss, M.J., Gray, S.M., and Cilia, M. (2013). Genomic and proteomic analysis of *Schizaphis graminum* reveals cyclophilin proteins are involved in the transmission of *Cereal yellow dwarf virus*. PLOS ONE *8*, e71620. https://doi.org/10.1371/journal.pone.0071620

Tholl, D., Chen, F., Petri, J., Gershenzon, J., and Pichersky, E. (2005). Two sesquiterpene synthases are responsible for the complex mixture of sesquiterpenes emitted from *Arabidopsis* flowers. Plant J. *42*, 757–771.

Thorpe, P., Cock, P.J., and Bos, J. (2016). Comparative transcriptomics and proteomics of three different aphid species identifies core and diverse effector sets. BMC Genomics *17*, 172. https://doi.org/10.1186/s12864-016-2496-6

Tian, L., Guo, E., Diao, Y., Zhou, S., Peng, Q., Cao, Y., Ling, E., and Li, S. (2010). Genome-wide regulation of innate immunity by juvenile hormone and 20-hydroxyecdysone in the *Bombyx* fat body. BMC Genomics *11*, 549. https://doi.org/10.1186/1471-2164-11-549

Tu, Z., Ling, B., Xu, D., Zhang, M., and Zhou, G. (2013). Effects of *Southern rice black-streaked dwarf virus* on the development and fecundity of its vector, *Sogatella furcifera*. Virol. J. *10*, 145. https://doi.org/10.1186/1743-422X-10-145

Ullman, D.E., Cho, J.J., Mau, R.F., Westcot, D.M., and Custer, D.M. (1992). A midgut barrier to *Tomato spotted wilt virus* acquisition by adult western flower thrips. Phytopathology *82*, 1333–1333.

Ullman, D.E., Westcot, D.M., Chenault, K.D., Sherwood, J.L., German, T.L., Bandla, M.D., Cantone, F., and Duer, H. (1995). Compartmentalization, intracellular transport, and autophagy of *Tomato spotted wilt* tospovirus proteins in infected thrips cells. Phytopathology *85*, 644–654.

van den Heuvel, J.F., Verbeek, M., and van der Wilk, F. (1994). Endosymbiotic bacteria associated with circulative transmission of *Potato leafroll virus* by *Myzus persicae*. J. Gen. Virol. *75*, 2559–2565. https://doi.org/10.1099/0022-1317-75-10-2559

van Molken, T., de Caluwe, H., Hordijk, C.A., Leon-Reyes, A., Snoeren, T.A., van Dam, N.M., and Stuefer, J.F. (2012). Virus infection decreases the attractiveness of white clover plants for a non-vectoring herbivore. Oecologia *170*, 433–444. https://doi.org/10.1007/s00442-012-2322-z

Vrkoslav, V., Muck, A., Cvacka, J., and Svatos, A. (2010). MALDI imaging of neutral cuticular lipids in insects and plants. J. Am. Soc. Mass Spectrom. *21*, 220–231. https://doi.org/10.1016/j.jasms.2009.10.003

Wan, G., Jiang, S., Wang, W., Li, G., Tao, X., Pan, W., Sword, G.A., and Chen, F. (2015). *Rice stripe virus* counters reduced fecundity in its insect vector by modifying insect physiology, primary endosymbionts and feeding behavior. Sci. Rep. *5*, 12527. https://doi.org/10.1038/srep12527

Wang, H., Xu, D., Pu, L., and Zhou, G. (2014). *Southern rice black-streaked dwarf virus* alters insect vectors' host orientation preferences to enhance spread and increase *Rice ragged stunt virus* co-infection. Phytopathology *104*, 196–201. https://doi.org/10.1094/PHYTO-08-13-0227-R

Wang, L., Tang, N., Gao, X., Guo, D., Chang, Z., Fu, Y., Akinyemi, I.A., and Wu, Q. (2016). Understanding the immune system architecture and transcriptome responses to *Southern rice black-streaked dwarf virus* in *Sogatella furcifera*. Sci. Rep. *6*, 36254. https://doi.org/10.1038/srep36254

Wang, X. (2003). Identification of a protein associated with circulative transmission of *Barley yellow dwarf virus* from cereal aphids, *Schizaphis graminum* and *Sitobion avenae*. Chin. Sci. Bull. *48*, 2083–2087. https://doi.org/10.1360/03wc0153

Wang, X.W., Luan, J.B., Li, J.M., Bao, Y.Y., Zhang, C.X., and Liu, S.S. (2010). *De novo* characterization of a whitefly transcriptome and analysis of its gene expression during development. BMC Genomics *11*, 400. https://doi.org/10.1186/1471-2164-11-400

Wang, Y., Mao, Q., Liu, W., Mar, T., Wei, T., Liu, Y., and Wang, X. (2014). Localization and distribution of *Wheat dwarf virus* in its vector leafhopper, *Psammotettix alienus*. Phytopathology *104*, 897–904. https://doi.org/10.1094/PHYTO-09-13-0251-R

War, A.R., Paulraj, M.G., Ahmad, T., Buhroo, A.A., Hussain, B., Ignacimuthu, S., and Sharma, H.C. (2012). Mechanisms of plant defense against insect herbivores. Plant Signal. Behav. *7*, 1306–1320. https://doi.org/10.4161/psb.21663

Webster, B.E.N. (2012). The role of olfaction in aphid host location. Physiol. Entomol. *37*, 10–18. https://doi.org/10.1111/j.1365-3032.2011.00791.x

Webster, C.G., Pichon, E., van Munster, M., Monsion, B., Deshoux, M., Gargani, D., Calevro, F., Jimenez, J., Moreno, A., Krenz, B., *et al.* (2018). Identification of plant virus receptor candidates in the stylets of their aphid vectors. J. Virol. e00432–00418. Epub ahead of print]. https://doi.org/10.1128/JVI.00432-18

Wenger, J.A., Cassone, B.J., Legeai, F., Johnston, J.S., Bansal, R., Yates, A.D., Coates, B.S., Pavinato, V.A., and Michel, A. (2017). Whole genome sequence of the soybean aphid, *Aphis glycines*. Insect Biochem. Mol. Biol. [Epub ahead of print]. https://doi.org/10.1016/j.ibmb.2017.01.005

Werner, B.J., Mowry, T.M., Bosque-Pérez, N.A., Ding, H., and Eigenbrode, S.D. (2009). Changes in green peach aphid responses to *Potato leafroll virus*-induced volatiles emitted during disease progression. Environ. Entomol. *38*, 1429–1438. https://doi.org/10.1603/022.038.0511

Westwood, J.H., Groen, S.C., Du, Z., Murphy, A.M., Anggoro, D.T., Tungadi, T., Luang-In, V., Lewsey, M.G., Rossiter, J.T., Powell, G., *et al.* (2013). A trio of viral proteins tunes aphid-plant interactions in *Arabidopsis thaliana*. PLOS ONE *8*, e83066. https://doi.org/10.1371/journal.pone.0083066

Whitfield, A.E., Ullman, D.E., and German, T.L. (2005). *Tomato spotted wilt virus* glycoprotein G(C) is cleaved at acidic pH. Virus Res. *110*, 183–186. https://doi.org/10.1016/j.virusres.2005.01.007

Whitfield, A.E., Rotenberg, D., Aritua, V., and Hogenhout, S.A. (2011). Analysis of expressed sequence tags from *Maize mosaic* rhabdovirus-infected gut tissues of *Peregrinus maidis* reveals the presence of key components of insect innate immunity. Insect Mol. Biol. *20*, 225–242. https://doi.org/10.1111/j.1365-2583.2010.01060.x

Whitfield, A.E., Falk, B.W., and Rotenberg, D. (2015). Insect vector-mediated transmission of plant viruses. Virology 479-480, 278–289. https://doi.org/10.1016/j.virol.2015.03.026

Whitham, S.A., Yang, C., and Goodin, M.M. (2006). Global impact: elucidating plant responses to viral infection. Mol. Plant Microbe Interact. *19*, 1207–1215. https://doi.org/10.1094/MPMI-19-1207

Woolston, C.J., Covey, S.N., Penswick, J.R., and Davies, J.W. (1983). Aphid transmission and a polypeptide are specified by a defined region of the *Cauliflower mosaic virus* genome. Gene *23*, 15–23.

Wu, W., Zheng, L., Chen, H., Jia, D., Li, F., and Wei, T. (2014a). Nonstructural protein NS4 of *Rice stripe virus* plays a critical role in viral spread in the body of vector insects. PLOS ONE *9*, e88636. https://doi.org/10.1371/journal.pone.0088636

Wu, Y., Davis, T.S., and Eigenbrode, S.D. (2014b). Aphid behavioral responses to virus-infected plants are similar despite divergent fitness effects. Entomol. Exp. Appl. *153*, 246–255. https://doi.org/10.1111/eea.12246

Xu, D., Zhong, T., Feng, W., and Zhou, G. (2016). Tolerance and responsive gene expression of *Sogatella furcifera* under extreme temperature stresses are altered by its vectored plant virus. Sci. Rep. *6*, 31521. https://doi.org/10.1038/srep31521

Xu, Y., Huang, L., Fu, S., Wu, J., and Zhou, X. (2012a). Population diversity of *Rice stripe virus*-derived siRNAs in three different hosts and RNAi-based antiviral immunity in *Laodelphgax striatellus*. PLOS ONE *7*, e46238. https://doi.org/10.1371/journal.pone.0046238

Xu, Y., Zhou, W., Zhou, Y., Wu, J., and Zhou, X. (2012b). Transcriptome and comparative gene expression analysis of *Sogatella furcifera* (Horváth) in response to *Southern rice black-streaked dwarf virus*. PLOS ONE *7*, e36238. https://doi.org/10.1371/journal.pone.0036238

Xu, Y., Wu, J., Fu, S., Li, C., Zhu, Z.R., and Zhou, X. (2015). *Rice stripe tenuivirus* nonstructural protein 3 hijacks the 26S proteasome of the small brown planthopper via direct interaction with regulatory particle non-ATPase subunit 3. J. Virol. *89*, 4296–4310. https://doi.org/10.1128/JVI.03055-14

Yang, N., Xie, W., Jones, C.M., Bass, C., Jiao, X., Yang, X., Liu, B., Li, R., and Zhang, Y. (2013). Transcriptome profiling of the whitefly *Bemisia tabaci* reveals stage-specific gene expression signatures for thiamethoxam resistance. Insect Mol. Biol. *22*, 485–496. https://doi.org/10.1111/imb.12038

Yang, X., Thannhauser, T.W., Burrows, M., Cox-Foster, D., Gildow, F.E., and Gray, S.M. (2008). Coupling genetics and proteomics to identify aphid proteins associated with vector-specific transmission of polerovirus (luteoviridae). J. Virol. *82*, 291–299.

Zamora, M., Méndez-López, E., Agirrezabala, X., Cuesta, R., Lavín, J.L., Sánchez-Pina, M.A., Aranda, M.A., and Valle, M. (2017). Potyvirus virion structure shows conserved protein fold and RNA binding site in ssRNA viruses. Sci. Adv. *3*, eaao2182. https://doi.org/10.1126/sciadv.aao2182

Zhang, F., Guo, H., Zheng, H., Zhou, T., Zhou, Y., Wang, S., Fang, R., Qian, W., and Chen, X. (2010). Massively parallel pyrosequencing-based transcriptome analyses of small brown planthopper (*Laodelphax striatellus*), a vector insect transmitting *Rice stripe virus* (RSV). BMC Genomics *11*, 303. https://doi.org/10.1186/1471-2164-11-303

Zhang, T., Luan, J.B., Qi, J.F., Huang, C.J., Li, M., Zhou, X.P., and Liu, S.S. (2012). Begomovirus-whitefly mutualism is achieved through repression of plant defences by a virus pathogenicity factor. Mol. Ecol. *21*, 1294–1304. https://doi.org/10.1111/j.1365-294X.2012.05457.x

Zhang, T., Feng, W., Ye, J., Li, Z., and Zhou, G. (2018). Metabolomic changes in *Sogatella furcifera* under *Southern rice black-streaked dwarf virus* infection and temperature stress. Viruses *10*, 344. https://doi.org/10.3390/v10070344

Zhang, Z., Zhang, P., Li, W., Zhang, J., Huang, F., Yang, J., Bei, Y., and Lu, Y. (2013). *De novo* transcriptome sequencing in *Frankliniella occidentalis* to identify genes involved in plant virus transmission and insecticide resistance. Genomics *101*, 296–305. https://doi.org/10.1016/j.ygeno.2013.02.005

Zheng, Y.X., Chen, C.C., and Jan, F.J. (2011). Complete nucleotide sequence of *Capsicum chlorosis virus* isolated from *Phalaenopsis* orchid and the prediction of the unexplored genetic information of tospoviruses. Arch. Virol. *156*, 421–432. https://doi.org/10.1007/s00705-010-0874-y

Zhou, F., Pu, Y., Wei, T., Wei, T., Liu, H., Deng, W., Wei, C., Ding, B., Omura, T., and Li, Y. (2007). The P2 capsid protein of the nonenveloped *Rice dwarf* phytoreovirus induces membrane fusion in insect host cells. Proc. Natl. Acad. Sci. U.S.A. *104*, 19547–19552. https://doi.org/10.1073/pnas.0708946104

Zhu, J., Jiang, F., Wang, X., Yang, P., Bao, Y., Zhao, W., Wang, W., Lu, H., Wang, Q., Cui, N., *et al.* (2017). Genome sequence of the small brown planthopper, *Laodelphax striatellus*. Gigascience *6*, 1–12. https://doi.org/10.1093/gigascience/gix109

Zhu, Y., Li, H., Bhatti, S., Zhou, S., Yang, Y., Fish, T., and Thannhauser, T.W. (2016). Development of a laser capture microscope-based single-cell-type proteomics tool for studying proteomes of individual cell layers of plant roots. Hortic. Res. *3*, 16026. https://doi.org/10.1038/hortres.2016.26

Ziebell, H., Murphy, A.M., Groen, S.C., Tungadi, T., Westwood, J.H., Lewsey, M.G., Moulin, M., Kleczkowski, A., Smith, A.G., Stevens, M., *et al.* (2011). *Cucumber mosaic virus* and its 2b RNA silencing suppressor modify plant-aphid interactions in tobacco. Sci. Rep. *1*, 187. https://doi.org/10.1038/srep00187

Ziegenhain, C., Vieth, B., Parekh, S., Hellmann, I., and Enard, W. (2018). Quantitative single-cell transcriptomics. Brief. Funct. Genomics *17*, 220–232. https://doi.org/10.1093/bfgp/ely009