Application of Genomics for Understanding Plant Virus-Insect Vector Interactions and Insect Vector Control

Navneet Kaur, Daniel K. Hasegawa, Kai-Shu Ling, and William M. Wintermantel

First and fourth authors: USDA-ARS, Crop Improvement and Protection Research, Salinas, CA 93905; second author: USDA-ARS, U.S. Vegetable Laboratory, Charleston, SC 29414; Boyce Thompson Institute, Cornell University, Ithaca, NY 14853; and third author: USDA-ARS, U.S. Vegetable Laboratory, Charleston, SC 29414.
Accepted for publication 7 July 2016.

ABSTRACT

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.

The relationships between plant viruses and their vectors have evolved over the millennia, and yet, studies on viruses began <150 years ago and investigations into the virus and vector interactions even more recently. The advent of next generation sequencing, including rapid genome and transcriptome analysis, methods for evaluation of small RNAs, and the related disciplines of proteomics and metabolomics offer a significant shift in the ability to elucidate molecular mechanisms involved in virus infection and transmission by insect vectors. Genomic technologies offer an unprecedented opportunity to examine the response of insect vectors to the presence of ingested viruses through gene expression changes and altered biochemical pathways. This review focuses on the interactions between viruses and their whitefly or thrips vectors and on potential applications of genomics-driven control of the insect vectors. Recent studies have evaluated gene expression in vectors during feeding on plants infected with begomoviruses, criniviruses, and tospoviruses, which exhibit very different types of virus-vector interactions. These studies demonstrate the advantages of genomics and the potential complementary studies that rapidly advance our understanding of the biology of virus transmission by insect vectors and offer additional opportunities to design novel genetic strategies to manage insect vectors and the viruses they transmit.

Next generation sequencing (NGS) includes a wide range of applications based on high throughput genome sequencing, genome resequencing, transcriptome profiling (RNA-Seq), small RNA analysis, and more. Numerous approaches can be used for whole-genome sequencing and the appropriate method will depend on the desired goal. If a reference sequence is already available, direct alignment can facilitate comparisons and improves the potential for gene identification. If reference sequences are not available, de novo assembly is required.

A very important aspect of NGS is the ability to examine gene expression and elements involved in the regulation of gene expression. RNA sequencing (RNA-Seq) can not only identify but, also, quantify levels of mRNA and small RNAs that may be involved in or result from regulation of gene expression (Anonymous 2016). The method is highly sensitive and can detect exceptionally low levels of individual RNAs as well as those that are highly expressed. As the technology associated with NGS has advanced, new opportunities to examine RNA have increased as well and can include the ability to look at RNA associated with specific organelles, such as mitochondria and ribosomes. Together, these NGS technologies provide a powerful toolbox for genetic analyses that determine whether an insect can transmit plant viruses, identify the factors that influence transmission efficiency, and understand the biology that drives the transmission of diverse plant viruses. Ultimately, these methods will facilitate the development of new genetic methods for managing both insect vectors and the plant viruses they transmit.

Viruses have been found in animals, plants, fungi, and bacteria. There were over 3,700 recognized viruses and viroids in 2015, and approximately a third of these are plant viruses (International Committee on Taxonomy of Viruses 2015). The number of viruses is steadily increasing because viruses have a high propensity to mutate and adapt to selection pressure. Most plant viruses damage agricultural crops through diseases they cause, resulting in reduced plant vigor, yield losses, unmarketable crops, or even plant death. Others can infect plants without exhibiting symptoms and, in some cases, may even coevolve with host plants (Fukuhara and Gibbs 2012; Koonin and Dolja 2012; Roossinck 2011, 2015). The majority of plant viruses are composed of single-stranded RNA with the virus genome in the sense orientation (i.e., members of families *Bromoviridae*, *Closteroviridae*, *Luteoviridae*, and *Potyviridae*). Other plant viruses possess single-stranded (ss)RNA genomes in an ambisense or antisense orientation (i.e., members of families *Bunyaviridae* and *Rhabdoviridae*), double-stranded (ds)RNA genomes (i.e., families *Reoviridae*, *Endornaviridae*), ssDNA genomes (family *Geminiviridae*), or dsDNA genomes (family *Caulimoviridae*).

The majority of plant viruses are transmitted by arthropods, nematodes, or fungi, although there are other means of transmission, such as through contaminated pollen, seed, or by plant-to-plant contact. Among the arthropod vectors of plant viruses, aphids, thrips, and whiteflies are, arguably, the most thoroughly examined with regard to their interactions with viruses. Virus transmission can be classified as either circulative or noncirculative, depending on whether or not the virus particles circulate throughout the body of the insect vector prior to transmission. Viruses that do not cross membranes within the vector are known collectively as non-circulative viruses, and these can be classified as either nonpersistent or semipersistent (Table 1). In contrast, circulative viruses are described as persistent circulative or persistent propagative (Table 1). Persistent viruses require ingestion of virus particles by the insects, with eventual transport of virus particles into the hemocoel and, subsequently, into the salivary glands, from which they are transmitted to new plants during feeding (Gray and Banerjee 1999). Nonpersistent viruses are acquired by insect vectors as they probe the host plant to

Corresponding author: W. M. Wintermantel;
E-mail address: bill.wintermantel@ars.usda.gov

http://dx.doi.org/10.1094/PHYTO-02-16-0111-FI

determine if it is a suitable food source or during the feeding process itself. They remain associated with insect mouthparts and usually remain transmissible for only a few minutes to, at most, a few hours following acquisition. There are many genera of nonpersistent viruses, although the best studied are those in the genus *Potyvirus* (Gray and Banerjee 1999; Pirone and Perry 2002). At the other end of the spectrum are persistent viruses, which, once acquired by the vector, are retained in a transmissible form for the life of the insect. Efficient transmission of persistent viruses requires longer acquisition feeding periods that can vary from hours to days and, frequently, have lengthy latent periods during which the virus cannot be transmitted. The best-studied persistent viruses are those in the family *Geminiviridae*, particularly within the genus *Begomovirus*. Viruses classified as semipersistent, such as those in *Closteroviridae*, encompass those with a wide range of transmission modes between the extremes of nonpersistent and persistent transmission, (Chen et al. 2011; Gray et al. 2014; Whitfield et al. 2015; Wintermantel 2016). In contrast to the relatively extensive information available on persistent and nonpersistent transmission, little information exists about the factors that facilitate virus retention and transmission for noncirculative semipersistent viruses.

Persistent circulative viruses, such as *Tomato yellow leaf curl virus* (TYLCV), are ingested by whiteflies (*Bemisia tabaci*) during feeding and are translocated across the wall of the epithelial cells within the gut into the hemolymph, from where they pass to the salivary glands and are egested into new plants through the salivary duct upon feeding (Czosnek et al. 2002). Viruses that replicate inside the insect are called persistent propagative viruses. These are uncommon among plant viruses but represent a highly adapted interaction between the virus and the vector and have been studied in the interactions between *Tomato spotted wilt virus* (TSWV) and its most common vector, the western flower thrips (*Frankliniella occidentalis)*. TSWV virions are acquired by thrips from infected plants, are ingested, and are replicated in the midgut epithelial cells. The virus particles are subsequently translocated to the muscle cells of the gut and, eventually, the salivary glands, where the virus also replicates. The virus is delivered into plants through saliva during feeding. Only larval thrips can acquire TSWV, but only adults can transmit the virus to plants (Whitfield et al. 2005).

Semipersistent or nonpersistent modes of virus transmission are both noncirculative and involve association of virus particles with the epicuticular lining of the insect foregut or stylet. This lining is cast off when the insect molts, thus eliminating the virus from the insect and preventing subsequent transmission; therefore, transmission can only persist for a limited time (Gray and Banerjee 1999). Viruses with nonpersistent transmission are usually lost rapidly upon acquisition by the feeding insects (Ammar et al. 1994; Gray and Banerjee 1999; Martin et al. 1997), whereas viruses with semipersistent transmission are retained for periods of several hours to several days. Considerable variation exists among insect species, subspecies, and some unique populations for virus retention times in insects as well as transmission efficiency (Maruthi et al. 2005; Wintermantel 2010; Wintermantel and Wisler 2006). Transmission is influenced by many factors, including virus titer in host plants (Ng et al. 2004; Wintermantel et al. 2008), environmental effects, and structural or physiological variation in source plants influencing insect feeding (Legg 1994; Wintermantel et al. 2016).

In this review, we focus on the applications of functional genomics using NGS technologies to enhance our knowledge on the molecular interactions of plant viruses with their insect vectors, virus detection in vectors, and implications for vector management. The use of functional genomic technologies, including RNA-Seq (Nagalakshmi et al. 2008) and small RNA sequencing to characterize gene expression, can clarify how insect vectors respond to ingestion of different types of plant viruses. These technologies offer a new era of functional genomics-based research, elucidating not only specific interactions but, also, pathways of regulated genes responding to the presence of a virus in a host plant or in the insect itself. The fundamental knowledge and thorough understanding of genes and pathways in an insect vector upon virus acquisition and transmission should lead to the design of novel genetic strategies such as RNA interference (RNAi) or the application of genome editing technologies to control an insect vector and the viruses it transmits (Gu and Knipple 2013).

CHARACTERIZATION OF GENETIC RELATIONSHIPS AMONG THE INSECT VECTORS OF PLANT VIRUSES

Differences in vector biology with regard to virus transmission have led to the classification of whitefly biotypes or subpopulations of other insects. While this is a valid biological trait, it is not always reflective of genetic variation within the insect vector populations. The emergence of molecular biology in the 1980s and 1990s led to the incorporation of sequence variation along with biological traits for the development of insect phylogenies. With regard to whiteflies, initial studies used differences in esterase patterns for the identification of whitefly biotypes from one another (Costa and Brown 1991). Later, the use of mitochondrial cytochrome oxidase I (*mtCOI*) gene sequences led to greater efficiency in the identification of population variation and rapid detection of the influx of new species or variants (Frohlich et al. 1996, 1999). This method is now widely used to differentiate species and subspecies level variants of sweetpotato whitefly (*Bemisia tabaci*) from one another. However, the use of a single gene for the determination of species variation limits reliability and accuracy of phylogenetic variation, particularly in areas where many related insect populations coexist. The *B. tabaci*

TABLE 1. Key characteristics of representative viruses for each of four primary modes of transmission

Transmission mode	Example (Genus)	Mode description	Retention time	Virus-vector example[a]
Persistent circulative	*Begomovirus* family *Geminiviridae*	Virus particles are ingested, translocated through gut wall into hemocoel, circulated to salivary glands, and transmitted to plants.	Life long	TYLCV-whitefly (*Bemisia tabaci*, MEAM1)
Persistent propagative	*Tospovirus* family	Virus particles are ingested, translocated through the gut wall into hemocoel, circulated and replicated in insect, and transmitted to plants.	Life long	TSWV-thrips (*Frankliniella occidentalis*)
Semipersistent	*Crinivirus* family	Virus particles are transiently attached to locations in the stylets, cibarium, or other mouthparts for several hours to days, then, released during insect feeding.	Hours to several days	LIYV-whitefly (*B. tabaci*, MEAM1)
Nonpersistent	*Potyvirus* family	Primarily associate with the epicuticular lining of the stylet or foregut for brief periods and quickly released to new plants during feeding.	Minutes to hours	PVY-aphid (*Myzus persicae*)

[a] TYLCV, *Tomato yellow leaf curl virus*; TSWV, *Tomato spotted wilt virus*; LIYV, *Lettuce infectious yellows virus*; PVY, *Potato virus Y.*

species complex is currently composed of 34 distinct entities that could, conceivably, be classified as species, based on both molecular and biological studies (Polston et al. 2014). However, improved criteria beyond those currently in use are needed for distinguishing variation, demonstrating a greater need for more specific knowledge of whitefly populations; and this is certain to be true for other insect vectors of plant viruses as well. For example, there are many *Bemisia* species and species variants present in sub-Saharan Africa that frequently exist as mixed populations in the field (Legg et al. 2014). These species are found in proximity to one another, even within the same field, but differ to varying degrees in host preference and, in some cases, ability to transmit important viruses within the region (Maruthi et al. 2005; Legg et al. 2014; Polston et al. 2014). There are documented differences among these populations for feeding preference on cassava and sweetpotato, two major crops produced in the region, and many subpopulations are related to one another (Boykin et al. 2012; Lapidot and Polston 2010; Legg et al. 2014). The availability of NGS provides improved opportunities to understand species variation among whitefly populations present in the region and globally and to move beyond what was possible through comparisons of single gene *mtCOI* sequences or biological differences (De Barro et al. 2011). This knowledge adds to our understanding of differentiating vector populations from one another and from related nonvector species.

APPLICATION OF NGS TECHNOLOGIES TO UNDERSTAND VECTOR-VIRUS INTERACTIONS

Begomovirus-whitefly interactions. Whiteflies and begomoviruses have been coevolving for millions of years. Over 300 begomoviruses (family *Geminiviridae*) have been described to date (International Committee on Taxonomy of Viruses 2015), many of which contribute to annual yield losses of billions of dollars while threatening global food security. Crops affected by begomoviruses include tomato, bean, squash, cassava, sweetpotato, cotton, and numerous others. These crops are impacted by infection with many viruses of huge economic impact, such as TYLCV, *Cotton leaf curl virus*, *Squash leaf curl virus*, *East African cassava mosaic virus*, and others. All begomoviruses are exclusively transmitted by whiteflies in a persistent and circulative manner. However, most of our current knowledge of whitefly-begomovirus interactions have come from the studies using TYLCV, which, along with closely related virus species, is an especially devastating virus affecting tomato production in most of the tropical and subtropical regions of the world. Several studies have explored the interactions between whiteflies and begomoviruses, which revealed two heat-shock proteins that can bind to the coat protein of TYLCV, suggesting their involvement during virus transmission (Ohnesorge and Bejarano 2009; Gotz et al. 2012). In addition, a whitefly bacterial symbiont-derived GroEL protein was found to interact with TYLCV virions and is implicated in protecting the virus during circulation within the whitefly host (Gottlieb et al. 2010; Morin et al. 2000). However, there remain many unanswered questions regarding whitefly genetics and physiology that drive virus acquisition, persistence, circulation, and transmission.

The first study that used NGS technologies came from a global transcriptome analysis of whiteflies (i.e., Middle East-Asia Minor 1 [MEAM1]) fed on *Tomato yellow leaf curl China virus* (TYLCCNV)-infected tobacco (Luan et al. 2011). In that study, whiteflies were reared on cotton, a nonhost plant for TYLCCNV, and were transferred to either healthy tobacco plants or TYLCCNV-infected tobacco plants and were allowed to feed for 24 h. Whiteflies were then transferred back to cotton plants and were fed for 5 days. The purpose of this was to reduce the changes in gene expression caused by exposure to healthy and infected tobacco plants (e.g., nutrition and plant volatiles). Therefore, any observed differential gene expression was expected to be associated with TYLCCNV acquisition by the whitefly. A single biological replicate was used for digital gene expression library preparation and Illumina sequencing, while a second replicate was used for quantitative polymerase chain reaction (PCR) validation of the predicted differentially expressed genes. In total, 1,606 genes were differentially expressed in viruliferous whiteflies; 298 of those genes were mapped to 157 pathways in the KEGG database (www.genome.jp/kegg/pathway.html). This suggested that acquisition of TYLCCNV can induce changes in the cell cycle, primary metabolism, and cellular and humoral immunity (Luan et al. 2011).

Regulation of several cell-cycle regulatory genes, as well as the downregulation of genes involved in lipid and amino acid metabolism, suggested that TYLCCNV appears to behave like a pathogen in whiteflies (Table 2) (Luan et al. 2011). This hypothesis was further supported by the upregulation of several genes involved in cellular and humoral immunity, including those that participate in autophagy, lysosome function, antimicrobial peptide production, coagulation, and melanization. The activation of an immune response could be interpreted as an evolved strategy for the whitefly to protect itself from the begomovirus, which, like a pathogen, circulates throughout the hemolymph of the insect, interacting with tissues and translocating across membranes. In contrast, numerous genes involved in signal transduction during antiviral immune responses, including toll, TGF-β, and mitogen-activated protein kinase pathways, were downregulated in whiteflies exposed to TYLCCNV, and genes that function in the apoptotic pathway were also downregulated (Table 2). In summary, the utilization of NGS technologies led to the hypothesis that the regulation of different immune pathways might be critical for virus persistence in the whitefly host (Luan et al. 2011).

Based on transcriptome analysis with the derived hypothesis that TYLCCNV can induce autophagy in whiteflies, the authors performed additional experiments to validate the implied physiological effects in vivo. Cytological staining and Western blotting, indeed, suggested that TYLCCNV can induce autophagy in the ovary and fat body tissues of whiteflies (Luan et al. 2011). Importantly, autophagy was only detected in viruliferous whiteflies 5 days after feeding on TYLCCNV-infected plants and was absent in whiteflies that fed on infected plants for 24 h, suggesting that autophagy occurs only after a certain incubation period inside whiteflies (Luan et al. 2011). This study illustrated how NGS technologies provided the background information necessary to develop testable hypotheses using traditional molecular biology methods to understand the complex relationship that has evolved between whiteflies and the begomoviruses they transmit (Table 2).

It is important to note that the Illumina data used in the studies described above was limited to a single biological replicate, largely due to the cost of sequencing at the time the experiments were conducted. However, as use of the technology is rapidly increasing, the cost of sequencing has continued to decrease. This leads to increased reliability of the data and effective validation of experiments through multiple biological replicates. Furthermore, it is critical to carefully regulate and standardize treatments and replications for valid comparisons. The time at which samples are collected and the conditions the samples are exposed to are both critical for standardization of experimental design among treatments and biological replications. This was essential for accurate identification of genes involved in persistence and circulation of the begomovirus in the whitefly. In this study, by allowing whiteflies to feed on TYLCCNV-infected tobacco for 1 day, followed by 5 days of feeding on a virus-free host plant, the authors aimed to reduce any secondary effects caused by differences between healthy and infected plants (e.g., nutrition and host volatiles). Therefore, any changes in gene expression between nonviruliferous and viruliferous whiteflies could be interpreted as effects induced by the begomovirus alone. The compromise with this experimental design is that any immediate changes in gene expression at the onset of begomovirus acquisition were not captured in the sequencing data. Furthermore, the consideration of potential secondary effects caused by differences between healthy and infected host plants would be important for understanding whitefly-begomovirus interactions in nature.

To partially fill this gap in knowledge, we (International Whitefly Genome Initiative 2015) performed transcriptome analysis on whiteflies that were allowed to feed on TYLCV-infected tomatoes. In this study, an isolate of TYLCV from South Carolina was propagated in 'Moneymaker' tomatoes. Whiteflies (MEAM1) were transferred to healthy tomato cuttings or TYLCV-infected cuttings and were allowed to feed for either 24, 48, or 72 h. Whiteflies were collected, were PCR-validated en masse to confirm virus acquisition at each time point, and were processed immediately for RNA-Seq analysis. Three biological replicates were performed for each treatment and time point. This differed from Luan et al. (2011) in that the new study addressed changes in whitefly gene expression at different time points corresponding to different virus acquisition access periods or the time during which the whiteflies were provided access to feeding on the virus-infected or healthy tomato plants. Although it is likely that whitefly gene expression was also influenced by secondary effects caused by physiological differences between healthy and virus-infected tomatoes, this study resembled a natural biological system. Furthermore, any changes in source plant physiology would be the result of virus infection of the acquisition host. The transcriptome was annotated using our whitefly reference genome (International Whitefly Genome Initiative 2015) and, among the 15,664 genes that were predicted, approximately 80 genes were differentially expressed across all three time points (Hasegawa et al. 2016; International Whitefly Genome Initiative 2015). Functional analysis revealed that many of the differentially expressed genes encoded either nucleic acid–binding proteins, receptors, or transporters or were involved in fatty acid metabolism, carbohydrate metabolism, or lysosome and protease activity.

Although indirect evidence suggests that begomovirus circulation within the whitefly is partially driven by receptor-mediated endocytic processes, a receptor is yet to be identified (Kollenberg et al. 2014; Rosen et al. 2015). Therefore, the identification of several upregulated receptors at 24 h provides a list of candidate genes that may be involved in facilitating the penetration and transport of the virus across the midgut and, possibly, into the primary salivary glands as well. It is possible that the regulation of these receptors is due to secondary effects by the host plants or may simply be explained as a response that is indirectly related to virus circulation. However, the utilization of NGS technologies has provided a list of candidate genes to address the hypothesis that begomovirus circulation in whiteflies is receptor-mediated.

Insect genes that were predicted to be involved in fatty acid metabolism were downregulated at 24 h, while genes involved in carbohydrate metabolism were downregulated at 72 h. This suggests that primary metabolism is regulated temporally across different exposure periods during feeding on a TYLCV-infected plant and further highlights the complex relationship between whiteflies and begomoviruses. Furthermore, this corroborates the work of Luan et al. (2011), which found acquisition of a begomovirus can induce changes in the regulation of primary metabolic pathways in the whitefly. Multiple downregulated genes that had lysosome and protease activity were also identified at 72 h. Interestingly, some of these genes have been implicated in virus transmission in other systems, including the mosquito-borne dengue virus (Kubo et al. 2012; Sim et al. 2012). In contrast, Luan et al. (2011) reported that most of the genes associated with lysosome function were upregulated rather than downregulated, which suggests a time-sensitive role for lysosomes during begomovirus acquisition and transmission. Examining transcriptome changes over multiple time points provides a more complete picture of how whitefly gene expression changes in response to begomovirus acquisition, because highly significant changes can occur over relatively short periods.

The application of functional genomics has provided a series of snapshots of the genetic networks that are influenced during transmission of begomoviruses by whiteflies. Together, these two studies examining the influence of begomovirus infection of host plants on whitefly gene expression complement each other in experimental design, and the results revealed dynamic temporal changes in gene expression during different stages of begomovirus interactions with whiteflies. The identification of whitefly genes involved in virus transmission may lead to targets for RNAi or other technologies as a management strategy for both whitefly and virus.

Crinivirus-whitefly interactions. In contrast to begomoviruses, very little is known about the mode by which semipersistent whitefly-transmitted viruses are acquired and retained in whiteflies to allow for virus transmission to new plants. Until recently, no studies had explored the potential for semipersistent viruses to

TABLE 2. Milestone studies on viruses and their interactions with vector insects using next generation sequencing (NGS)

Virus	Vector	NGS technique	Finding	Reference
Tomato yellow leaf curl China virus	Whitefly, *Bemisia tabaci*, MEAM1	Digital gene expression on viruliferous whiteflies	Downregulation of genes involved in lipid and amino acid metabolism. Upregulation of several genes involved in cellular and humoral immunity, including autophagy, lysosome function, antimicrobial peptide production, coagulation, and melanization. Downregulation of genes involved in antiviral immune responses, including toll, TGF-β, and mitogen-activated protein kinase pathways.	Luan et al. 2011
Tomato chlorosis virus	Whitefly, *Bemisia tabaci*, MEAM1	RNA-Seq on viruliferous whiteflies at three different time points 24, 48, and 72 h	Significantly higher numbers of differentially regulated genes at 24 and 72 h compared with few differences at 48 h. Significant number of glucose transporter and glucosidase family genes were differentially regulated in viruliferous whiteflies.	Chen et al. submitted
Tomato spotted wilt virus	Western flower thrips, *Frankliniella occidentalis*	RNA-Seq on viruliferous thrips at different life stages	Downregulation of genes related to ribosomes, amino acid metabolism, and carbohydrate in viruliferous thrips. Upregulation of genes associated with inhibition of virus replication.	Zhang et al. 2013b

influence gene expression in their vector whiteflies. It is important to understand whether semipersistent viruses are merely transmissible due to fortuitous association with factors that bind them long enough to allow transmission. Alternatively, the virus and vector may have coevolved such that the presence of virus would influence gene expression in the vector itself. Yet another possibility would be a virus-induced defense response leading to gene expression changes in the insect vector that may influence virus retention and vector transmission. Unlike members of the genus *Begomovirus*, the semipersistent virus members of the genus *Crinivirus* do not circulate within the whitefly vector but can remain within the mouthparts of the whitefly for periods ranging from a day to up to two weeks, depending on the virus and the whitefly vector (Wintermantel 2010; Wintermantel and Wisler 2006; Wisler and Duffus 2001). Furthermore, research showed criniviruses do actively interact with components in the mouthparts of the whitefly. Studies using in vitro virus acquisition demonstrated that the crinivirus *Lettuce infectious yellows v*irus (LIYV) specifically associates with the cibarium or anterior foregut of whiteflies capable of transmitting the virus but not in nonvector whiteflies (Chen et al. 2011). Furthermore, feeding vector whiteflies with a solution containing antibodies against the minor coat protein of LIYV interfered with transmission, demonstrating a role for the minor coat protein in transmission and, likely, in the association of the virus with the cibarium or anterior foregut of the whitefly (Tian et al. 1999; Stewart et al. 2010).

Tomato chlorosis virus (ToCV) (genus *Crinivirus*, family *Closteroviridae*) is a semipersistently transmitted virus that can cause a decline in plant vigor and reduce fruit yield in economically important vegetable crops, including tomato, potato, tomatillo, and sweet pepper (Tzanetakis et al. 2013). ToCV affects 24 plant species from seven different families (Wintermantel and Wisler 2006) and has a broad range of vectors, including *B. tabaci* New World 1, MEAM1, and MED (also known as biotypes A, B, and Q, respectively) (Wisler et al. 1998a, b; Navas-Castillo et al. 2000), as well as the greenhouse whitefly (*Trialeurodes vaporariorum*) and the banded-wing whitefly (*T. abutilonea*) (Wisler et al. 1998a, b). Transmission efficiency and persistence in the vector differs among species and haplotypes (Wintermantel and Wisler 2006).

To determine the whitefly gene expression profile upon ToCV acquisition, we examined the global transcriptional changes that occur in *B. tabaci* MEAM1 (biotype B) during its interaction with the semipersistent ToCV using high-throughput Illumina paired-end RNA-Seq technology (Chen et al. submitted; International Whitefly Genome Initiative 2015). In all, 989 genes were differentially expressed in ToCV-whiteflies at 24 h and 210 at 72 h, while only 11 genes were differentially expressed at 48 h (Table 2). This demonstrated temporally influenced gene expression in whiteflies due to virus acquisition from ToCV-infected source plants and that higher numbers of genes exhibited differences in expression within the first 24 h of feeding compared with whiteflies fed on virus-free controls. In addition, a different set of genes showed altered regulation at 72 h than at 24 h (Kaur et al. 2016). ToCV retention in the vector also subsides at approximately 72 h following virus acquisition (Wintermantel and Wisler 2006). Included among those differentially expressed genes, gene families, or pathways that may be involved in ToCV acquisition and transmission were glucose transporter and glucosidases (Chen et al. submitted; International Whitefly Genome Initiative 2015). The expression of the glucose transporter gene *GLUT3* was also found significantly increased in H9 lymphocytic cells upon *Human immunodeficiency virus* (HIV) infection. Other studies have implicated sugar transporter genes in virus-host interactions such as *Feline leukemia virus C* (Huang et al. 2015; Tailor et al. 1999). Alpha-glucosidase inhibitors were shown to act as antiviral agents against HIV and hepatitis B virus (Walker et al. 1987). Further studies will be needed to clarify which genes or biochemical pathways are directly influenced by the presence of the virus and which may be secondarily affected.

Tospovirus-thrips interactions. As with whiteflies, many species of thrips damage plants directly by feeding and a few by serving as vectors of plant viruses (Morse and Hoddle 2006). The western flower thrips *Frankliniella occidentalis* causes severe damage to agricultural crops through direct feeding damage as well as by transmitting tospoviruses, such as *Tomato spotted wilt virus* (TSWV) (Nagata et al. 1999). Like begomoviruses, tospoviruses are circulative in their vectors. In contrast, however, tospoviruses also replicate within their vector and must be acquired during the larval stages in order to be transmitted by the adult thrips (Hogenhout et al. 2008; Nagata et al. 1999). This demonstrates what is likely an even higher level of interaction between the virus and its insect vector when compared with whitefly-transmitted viruses. Using high-throughput Illumina sequencing, Zhang et al. (2013b) developed de novo transcriptome sequencing data for western flower thrips and investigated the changes in gene expression between virus-free western flower thrips reared on healthy plants and viruliferous thrips reared on TSWV-infected tomato plants (Table 2). The authors examined not only the adult *F. occidentalis* but also the first instar, second instar, prepupae, and pupae within the same

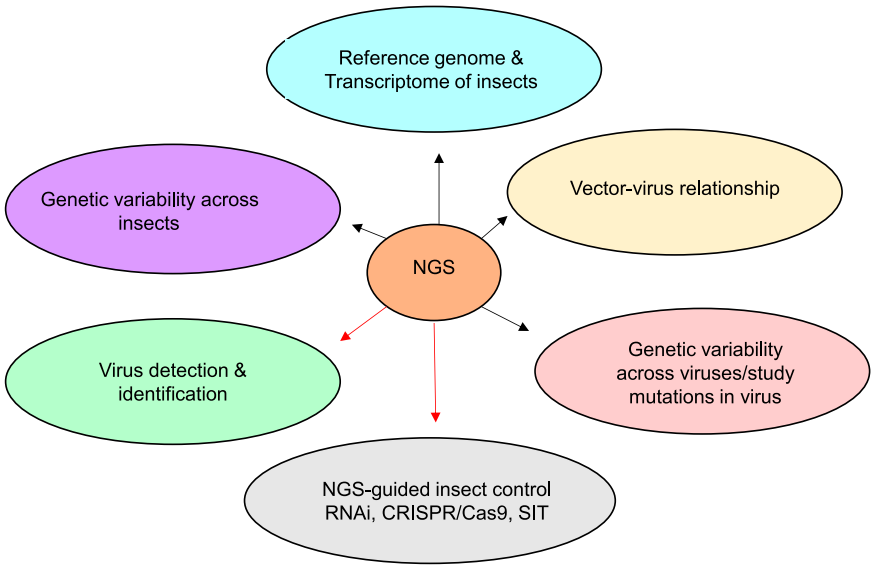

Fig. 1. Fundamental and applied applications of next generation sequencing (NGS) technologies in insect molecular biology, virus transmission, and virology. Black and red arrows represent, respectively, fundamental and applied aspects of NGS.

study, allowing comparison of differential gene expression associated with virus infection across insect developmental stages (Zhang et al. 2013b). The results identified 59,932 unigenes, of which 36,339 were annotated using public databases. Infection of western flower thrips by TSWV led to downregulation of genes related to ribosomes, amino acid metabolism, and carbohydrate metabolism compared with virus-free thrips and indicated that the TSWV infection caused decreased protein synthesis and amino acid and carbohydrate metabolism in the infected insects (Table 2) as well as altered cellular and immune responses in western flower thrips. The authors used results of transcriptome analysis to speculate that high levels of mucin expression may contribute to infection, replication, and diffusion of TSWV within thrips. Genes often associated with inhibition of virus replication were upregulated, suggesting that TSWV could activate an immune response in *F. occidentalis* upon infection (Zhang et al. 2013b).

Tissue-specific transcriptome studies with *F. occidentalis*. To evaluate gene expression in specific organs of thrips in response to TSWV feeding, Stafford-Banks et al. (2014) characterized the transcriptome of the salivary gland of male and female *F. occidentalis* in response to the presence and absence of TSWV. Results of transcriptome analysis revealed numerous genes implicated in plant detoxification or defense inhibition, sugar metabolism, and plant cell-wall degradation. The transcriptome data from the salivary gland of thrips enriches the functional genomics tools available for the further study of deep interactions of thrips-plant and thrips-virus relationships. Furthermore, this information is useful to develop novel RNAi approaches for the management of *F. occidentalis* and, thus, the tospoviruses. It should be noted that examination of gene expression in specific tissues that have not been analyzed previously may result in the identification of high percentages of unknown proteins.

NGS for virus identification in plant and animal (insect) systems. Over the last several years, the development and application of NGS technologies have enabled the rapid and efficient discovery of known and unknown viruses and viroids in plants and animals (Roossinck et al. 2015; Webster et al. 2015; Wu et al. 2015). The single greatest advantage in using the NGS technologies for virus discovery is that there is no requirement for a priori knowledge of the virus to be identified. Furthermore, sensitivity of the NGS analysis is extremely high. NGS technologies can result in rapid determination of an entire virus genome sequence or even confirm the presence of multiple viruses in a mixed infection (Al Rwahnih et al. 2013). There is even a possibility one can characterize, to a limited degree, the genetic diversity in a virus population from a single analysis. In this section, we provide a brief overview on the use of NGS technologies for virus discovery and identification in both plants and insects. In contrast with traditional methods for virus identification, the entire process from extraction of nucleic acid to analysis of results can take as little as a few weeks.

Several viruses have been identified from plants, using diverse plant nucleic acids for NGS analysis, including mRNA (Adams et al. 2009), dsRNA (Al Rwahnih et al. 2009, 2013; Coetzee et al. 2010), and small RNA (sRNA) (Hagen et al. 2011; Kreuze et al. 2009; Li et al. 2012; Navarro et al. 2009) extracted from plants. DNA viruses (e.g., geminiviruses) also produce sRNA in plants; therefore, sRNA deep sequencing is able to identify DNA viruses (Chen et al. 2015) as well as viroids (Li et al. 2012; Navarro et al. 2009; Wu et al. 2012).

In parallel with virus discovery in plants, during the last decade, NGS technologies have also been used successfully for virus discovery of human and animal viruses in the arthropod vectors (Bichaud et al. 2014). Although many previously known as well as novel viruses have been identified in insect species through the use of NGS technologies (Liu et al. 2015; Nouri et al. 2016; Webster et al. 2015), there remains very limited application of NGS for the discovery of plant viruses that are harbored in or transmitted by insect vectors such as aphids, mealybugs, leafhoppers, thrips, whiteflies, or others. sRNA extracts were proven to be a suitable nucleic acid material for NGS and virus identification from insect cell cultures (Wu et al. 2010). Another material used is viral RNA extracted from a suspension enriched for virus particles through virus purification in insect tissues. Metagenomic analysis on whiteflies was able to identify typical begomoviruses, using sequencing of these virus-enriched nucleic acid extracts (Ng et al. 2011). Using such sample preparation, several DNA and RNA plant viruses have been successfully identified in whitefly (Rosario et al. 2014). Moreover, a previously unknown whitefly-transmitted carlavirus, closely related to *Cowpea mild mottle virus*, was identified in whitefly through metagenomic analysis, which was followed by confirmation of its presence in local native plants (Rosario et al. 2014). These studies demonstrate the power of using the NGS technology for novel virus discovery in insect vectors.

Bioinformatics pipeline development to facilitate virus discovery and identification. The increasing access to NGS technologies provides a powerful new resource for identification of known and unknown viruses and virus variants in plant and animal systems. Despite its many advantages, the bioinformatics analysis of massive NGS datasets for sequence assembly and analysis is still not available in the ordinary plant pathology research laboratory. As a result, such analysis usually requires specialized skilled personnel or collaboration with an experienced bioinformatician. With the recent development of two simple and effective bioinformatics pipelines, including VirFind (http://VirFind.org/) (Ho and Tzanetakis 2014) and VirusDetect (http://bioinfo.bti.cornell.edu/tool/VirusDetect/) (Li et al. 2012), it is becoming feasible for laboratories without their own internal bioinformatics capacity to adapt such powerful technology. The VirFind program was designed for virus identification using longer read sequences, such as those generated with RNA-Seq (DiBello et al. 2015; Ho et al. 2016). The VirusDetect program (Li et al. 2012) is designed not only for the assembly of sRNA sequences but, also, for assembly of RNA-Seq datasets from both plants and animals for virus identification. This program has been proven effective in the identification of numerous viruses and viroids (Li et al. 2012, 2015a, b, c; Padmanabhan et al. 2014, 2015).

IMPLICATIONS

The ability to decipher genome sequences is providing new insights into the study of not only plant viruses and their interactions with host plants but, importantly, the intricate interactions that allow a virus to be transmitted by an insect vector. By decoding gene functions and identifying the types of genes and associated pathways that are up- or down-regulated during virus transmission, it is possible to gain a much broader understanding of this critical process impacting virus emergence and epidemiology. NGS provides a wealth of sequence information through the construction of high-quality reference genomes of many insects (Fig. 1). To date, these include but are not limited to *Drosophila melanogaster* (fruit fly), *Apis mellifera* (honey bee), *Bombyx mori* (Silkworm), *Tribolium castaneum* (red flour beetle), *Acyrthosiphon pisum* (pea aphid), *Aedes aegypti* (yellow fever mosquito), and *Bemisia tabaci* MEAM1 (sweetpotato whitefly) that are available in the public domain (Adams et al. 2000; Honeybee Genome Sequencing Consortium 2006; International Aphid Genomics Consortium 2010; International Whitefly Genome Initiative 2015; Nene et al. 2007; Tribolium Genome Sequencing Consortium 2008; Xia et al. 2004).

Consequently, there is a rapid surge of studies utilizing high-throughput and sensitive genomics techniques, including RNA-Seq and sRNA sequencing, to understand vector-virus relationships (Fig. 1). Included in these studies is the application of NGS for the identification of unknown viruses or other pathogens that may cause disease in plants but are carried by insects and may even lead to the identification of viruses that are unexpected, symptomless, or acting synergistically with known viruses (Fig. 1). Information on

how insect vectors respond to the presence of plant viruses through changes in gene expression resulting from feeding on virus infected plants or purified virions or virus proteins can contribute to determining why some insect species can transmit a virus while related species cannot. Furthermore, such information can lead to the identification of new molecular markers for reliable identification of vector insect species or subspecies (Fig. 1).

Due to the heavy use of insecticides, insect pests, including vectors of plant viruses, are developing resistance to widely used insecticides. These pesticides often have deleterious effects on nontarget insects as well as the environment. Therefore, alternate and highly effective methods for insect control are needed. RNAi is an important and rapidly emerging approach that can be used to enhance or suppress expression of select genes and has the potential to effectively and reliably replace insecticides for control of insect pests (Baum et al. 2007; Gu and Knipple 2013). The method is highly specific to desired target sequences, such that RNAi can be designed to disrupt functioning of specific target genes in a particular insect but not harm other insects, such as bees or butterflies. Thus, RNAi can be considered a more environmentally friendly approach for insect control than traditional pesticide use. The number of successful reports of RNAi for control of insect pests is steadily increasing, with demonstrated success against both human- and plant-feeding insects. These include but are certainly not limited to *Aedes aegypti*, the mosquito responsible for carrying malaria and other human diseases, *Bemisia tabaci*, one of the most important vectors of plant viruses in tropical and subtropical regions of the world, and *Diabrotica virgifera virgifera* LeConte, the western corn rootworm (Baum et al. 2007; Ghanim et al. 2007; Pridgeon et al. 2008; Upadhyay et al. 2011; Zhang et al. 2013a; Zotti and Smagghe 2015).

In addition to RNAi approaches for insect control, the vast amount of information on genes and gene expression generated through RNA-Seq can be utilized to select candidate genes for a method called sterile insect technology (SIT) that is being used increasingly to reduce pest insect populations (Alphey et al. 2010). The SIT technique is species-specific, effective, safe, and sustainable and is based on the release of a large number of sterilized male insects into a population. The method has been used effectively against *Drosophila melanogaster* and *Aedes aegypti*. Using this method, a gene is inserted by genetic modification into male insects. Although the male insects are not sterile, they contain a dominant lethal gene that results in the mortality of female progeny. The strategy is to release enough transgenic male mosquitoes in the natural population that they will mate with the females in the wild, resulting in large numbers of nonviable female progeny. This variant of SIT is known as "release of insects carrying a dominant lethal" (RIDL) (Phuc et al. 2007; Thomas et al. 2000). The RIDL system was demonstrated in *Drosophila melanogaster* and *Aedes aegypti* through expression of tetracycline-repressible transactivator fusion proteins and a tetracycline-repressible activator variant (tTAV), respectively (Thomas et al. 2000). In this case, the pest control gene produces a protein called tTAV that drives the expression of required cellular genes that have tetracycline-repressive elements in their promoter regions. However, the tTAV is optimized to specifically function in insect cells (Oxitec Ltd., Oxford). In a natural system, with no tetracycline, the genetically modified insects will produce tTAV protein. Under the expression of tTAV gene, the necessary cellular genes will be repressed because the tTAV protein binds to the tetracycline-responsive elements present in the promoter regions of genes, resulting in death of the progeny insects. It has been demonstrated that these genetically modified insects have no harmful effects on other animals nor are there any toxic proteins produced in them (Oxitec Ltd.). The technology has been used effectively to control the mosquito *Aedes aegypti* in order to reduce spread of malaria and dengue fever (Wise de Valdez et al. 2011). SIT has also been used successfully for the control of important plant insect pests around the world, including the pink bollworm (*Pectinophora gossypiella*), and the Mediterranean fruit fly (*Ceratitis capitata*) (Morrison et al. 2012; Leftwich et al. 2014). The SIT and RIDL approaches are examples of technologies that will benefit from the identification of target genes through NGS that are more lethal to insects and more environmentally suitable.

Resequencing is another popular NGS application and is now used routinely to determine the genomic variations of a sample in relation to a common reference sequence. In resequencing, the generated sequence is aligned to the reference sequence and mined for single nucleotide polymorphisms (SNPs) and copy number variations as well as genomic rearrangements, insertions, and deletions. The reference genome of *B. tabaci* MEAM1, which is one of the most important vectors of plant viruses worldwide, was recently completed (International Whitefly Genome Initiative 2015). The availability of this reference genome will facilitate resequencing at the genome level, using whole-genome sequencing or utilizing more cost-effective methods, such as genotyping by sequencing from numerous whitefly species, including but not limited to *B. tabaci* MEAM1, MED, Cassava whitefly (SSA1), the greenhouse whitefly (*T. vaporariorum*), and others. Differences in the genome, particularly SNPs, among distinct whitefly species across the world, may provide base knowledge for developing a SNP chip based on diverse alleles that could be utilized not only to identify whitefly species but, also, to clarify differences among cryptic whitefly species (DeBarro et al. 2011; Dinsdale et al. 2010; Xu et al. 2010). Such a chip could aid in taxonomic classification and provide knowledge of genetic differences among vector whiteflies. This might clarify how these *B. tabaci* cryptic species, particularly MEAM1 and MED, have established so effectively throughout tropical and subtropical climates and why they are so highly adapted for transmission of plant viruses. Furthermore, as noted previously, the availability of high-quality genome sequence will facilitate improved understanding of the genetic relationships among the ever-increasing number of whitefly vectors and nonvectors of plant viruses. This, in turn, will enhance opportunities to develop novel management strategies with sufficiently narrow or broad applicability, as desired, using RNAi or clustered regularly interspaced short palindromic repeats or additional emerging technologies.

The number of high-quality reference genomes of insects has been increasing rapidly as a result of a directed project to sequence the genomes of 5,000 arthropod species (i5K Consortium 2013). The outcome of this should be a wealth of genomic information including not only sequences but, also, gene expression on numerous insect pests. This can be coupled with related technologies, including functional genomics, proteome analysis, and metabolome analysis to provide an even broader portrait of the intricacies that allow viruses to be transmitted by insects and to understand the physiological and biochemical biology of the insect. As this information becomes available, methods similar to those described herein can be applied and, undoubtedly, numerous additional strategies will be developed and advanced for control of insect vectors of plant pathogens and pests of agricultural crops.

ACKNOWLEDGMENTS

This work was supported in part by a USDA-ARS Area-Wide project grant to K.-S. Ling and W. M. Wintermantel, and a grant from the U.S. Agency for International Development (USAID) Feed-the-Future program (58-0210-3-012) coordinated by the USDA-ARS Office of International Research Programs.

LITERATURE CITED

Adams, I., Glover, R., Monger, W., Mumford, R., Jackeviciene, E., Navalinskiene, M., Samuitiene, M., and Boonham, N. 2009. Next-generation sequencing and metagenomic analysis: A universal diagnostic tool in plant virology. Mol. Plant Pathol. 10:537-545.

Adams, M. D., Celniker, S. E., Holt, R. A., Evans, C. A., Gocayne, J. D., Amanatides, P. G., Scherer, S. E., Li, P. W., Hoskins, R. A., Galle, R. F., George, R. A., Lewis, S. E., Richards, S., Ashburner, M., Henderson, S. N.,

Sutton, G. G., Wortman, J. R., Yandell, M. D., Zhang, Q., Chen, L. X., Brandon, R. C., Rogers, Y. H., Blazej, R. G., Champe, M., Pfeiffer, B. D., Wan, K. H., Doyle, C., Baxter, E. G., Helt, G., Nelson, C. R., Gabor, G. L., Abril, J. F., Agbayani, A., An, H. J., Andrews-Pfannkoch, C., Baldwin, D., Ballew, R. M., Basu, A., Baxendale, J., Bayraktaroglu, L., Beasley, E. M., Beeson, K. Y., Benos, P. V., Berman, B. P., Bhandari, D., Bolshakov, S., Borkova, D., Botchan, M. R., Bouck, J., Brokstein, P., Brottier, P., Burtis, K. C., Busam, D. A., Butler, H., Cadieu, E., Center, A., Chandra, I., Cherry, J. M., Cawley, S., Dahlke, C., Davenport, L. B., Davies, P., de Pablos, B., Delcher, A., Deng, Z., Mays, A. D., Dew, I., Dietz, S. M., Dodson, K., Doup, L. E., Downes, M., Dugan-Rocha, S., Dunkov, B. C., Dunn, P., Durbin, K. J., Evangelista, C. C., Ferraz, C., Ferriera, S., Fleischmann, W., Fosler, C., Gabrielian, A. E., Garg, N. S., Gelbart, W. M., Glasser, K., Glodek, A., Gong, F., Gorrell, J. H., Gu, Z., Guan, P., Harris, M., Harris, N. L., Harvey, D., Heiman, T. J., Hernandez, J. R., Houck, J., Hostin, D., Houston, K. A., Howland, T. J., Wei, M. H., Ibegwam, C., Jalali, M., Kalush, F., Karpen, G. H., Ke, Z., Kennison, J. A., Ketchum, K. A., Kimmel, B. E., Kodira, C. D., Kraft, C., Kravitz, S., Kulp, D., Lai, Z., Lasko, P., Lei, Y., Levitsky, A. A., Li, J., Li, Z., Liang, Y., Lin, X., Liu, X., Mattei, B., McIntosh, T. C., McLeod, M. P., McPherson, D., Merkulov, G., Milshina, N. V., Mobarry, C., Morris, J., Moshrefi, A., Mount, S. M., Moy, M., Murphy, B., Murphy, L., Muzny, D. M., Nelson, D. L., Nelson, D. R., Nelson, K. A., Nixon, K., Nusskern, D. R., Pacleb, J. M., Palazzolo, M., Pittman, G. S., Pan, S., Pollard, J., Puri, V., Reese, M. G., Reinert, K., Remington, K., Saunders, R. D., Scheeler, F., Shen, H., Shue, B. C., Sidén-Kiamos, I., Simpson, M., Skupski, M. P., Smith, T., Spier, E., Spradling, A. C., Stapleton, M., Strong, R., Sun, E., Svirskas, R., Tector, C., Turner, R., Venter, E., Wang, A. H., Wang, X., Wang, Z. Y., Wassarman, D. A., Weinstock, G. M., Weissenbach, J., Williams, S. M., Woodage, T., Worley, K. C., Wu, D., Yang, S., Yao, Q. A., Ye, J., Yeh, R. F., Zaveri, J. S., Zhan, M., Zhang, G., Zhao, Q., Zheng, L., Zheng, X. H., Zhong, F. N., Zhong, W., Zhou, X., Zhu, S., Zhu, X., Smith, H. O., Gibbs, R. A., Myers, E. W., Rubin, G. M., and Venter, J. C. 2000. The genome sequence of *Drosophila melanogaster.* Science 287:2185-95.

Al Rwahnih, M., Ashita, D., Anderson, M. M., Rowhani, A., Uyemoto, J. K., and Sudarshana, M. R. 2013. Association of a DNA virus with grapevines affected by red blotch disease in California. Phytopathology 103:1069-1076.

Al Rwahnih, M., Daubert, S., Golino, D., and Rowhani, A. 2009. Deep sequencing analysis of RNAs from a grapevine showing Syrah decline symptoms reveals a multiple virus infection that includes a novel virus. Virology 387:395-401.

Alphey, L., Benedict, M., Bellini, R., Clark, G. G., Dame, D. A., Service, M. W., and Dobson, S. L. 2010. Sterile-insect methods for control of mosquito-borne diseases: An analysis. Vector-Borne Zoonot. 10:295-311.

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 84:1054-60.

Anonymous. 2016. An introduction to next generation sequencing technology. Pub. No. 770-2012-008, Illumina, Inc., San Diego, CA. http://www.illumina.com/content/dam/illumina-marketing/documents/products/illumina_sequencing_introduction.pdf.

Baum, J. A., Baum, T. B., Clinton, W., Heck, G. R., Feldmann, P. O., Ilagan, S., Johnson, G., Plaetinck, T., Munyikwa, M., Pleau, T., and Vaughn, J. R. 2007. Control of coleopteran insect pests through RNA interference. Nat. Biotechnol. 25:1322-1326.

Bichaud, L., De Lamballerie, X., Alkan, C., Izri, A., Gould, E. A., and Charrel, R. N. 2014. Arthropods as a source of new RNA viruses. Microb. Pathog. 77:136-141.

Boykin, L. M., Armstrong, K. F., Kubatko, L., and De Barro, P. 2012. Species delimitation and global biosecurity. Evol Bioinform 8:1-37.

Chen, A. Y. S., Walker, G. P., Carter, D., and Ng, J. C. K. 2011. A virus capsid component mediates virion retention and transmission by its insect vector. Proc. Natl. Acad. Sci. USA 108:16777-16782.

Chen, S., Huang, Q., Wu, L., and Qian, Y. 2015. Identification and characterization of a maize-associated mastrevirus in China by deep sequencing small RNA populations. Virol. J. 12:156.

Coetzee, B., Freeborough, M. J., Maree, H. J., Celton, J. M., Rees, D. J., and Burger, J. T. 2010. Deep sequencing analysis of viruses infecting grapevines: Virome of a vineyard. Virology 400:157-163.

Costa, H. S., and Brown, J. K. 1991. Variation in biological characteristics and in esterase patterns among populations of *Bemisia tabaci* (Genn.) and the association of one population with silverleaf symptom development. Entomol. Exp. Appl. 61:211-219.

Czosnek, H., Ghanim, M., and Ghanim, M. 2002. The circulative pathway of begomoviruses in the whitefly vector *Bemisia tabaci*—Insights from studies with *Tomato yellow leaf curl virus*. Ann. Appl. Biol. 140:215-231.

De Barro, P. J., Liu, S. S., Boykin, L. M., and Dinsdale, A. B. 2011. *Bemisia tabaci*: A statement of species status. Annu. Rev. Entomol. 56:1-19.

Di Bello, P. L., Ho, T., and Tzanetakis, I. E. 2015. The eveloution of emaraviruses is becoming more complex: Seven segments identified in the causal agent of rose rosette disease. Virus Res. 210:241-244.

Dinsdale, A., Cook, L., Riginos, C., Buckley, Y. M., and De Barro, P. 2010. Refined global analysis of *Bemisia tabaci* (Hemiptera: Sternorrhyncha: Aleyrodoidea: Aleyrodidae) mitochondrial cytochrome oxidase 1 to identify species level genetic boundaries. Ann. Entomol. Soc. Am. 103:196-208.

Frohlich, D. R., Brown, J. K., Bedford, I., and Markham, P. G. 1996. Mitochondrial 16S ribosomal subunit as a molecular marker in Bemisia, and implications for population variability. Pages 143-145 in: Bemisia 1995: Taxonomy, Biology, Damage Control, and Management. Intercept, Ltd., Andover, U.K.

Frohlich, D. R., Torres-Jerez, I. I., Bedford, I. D., Markham, P. G., and Brown, J. K. 1999. A phylogeographical analysis of the *Bemisia tabaci* species complex based on mitochondrial DNA markers. Mol. Ecol. 8:1683-1691.

Fukuhara, T., and Gibbs, M. J. 2012. Family Endornaviridae. Pages 519-521 in: Virus Taxonomy: Classification and Nomenclature of Viruses, Ninth Report of the International Committee on Taxonomy of Viruses. A. M. Q. King, M. J. Adams, E. B. Carstens, and E. J. Lefkowitz, eds. Elsevier/Academic Press.

Ghanim, M., Kontsedalov, S., and Czosnek, H. 2007. Tissue-specific gene silencing by RNA interference in the whitefly *Bemisia tabaci* (Gennadius). Insect Biochem. Mol. 37:732-738.

Gottlieb, Y., Zchori-Fein, E., Mozes-Daube, N., Kontsedalov, S., Skaljac, M., Brumin, M., Sobol, I., Czosnek, H., Vavre, F., and Fleury, F. 2010. The transmission efficiency of *Tomato yellow leaf curl virus* by the whitefly *Bemisia tabaci* is correlated with the presence of a specific symbiotic bacterium species. J. Virol. 84:9310-9317.

Gotz, 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.

Gray, S., Cilia, M., and Ghanim, M. 2014. Nonpropagativevirus transmission: An orchestra of virus-, insect-, and plant-derived instruments. Adv. Virus Res. 89:141-199.

Gray, S.M., and Banerjee, N. 1999. Mechanisms of arthropod transmission of plant and animal viruses. Microbiol. Mol. Biol. R. 83:128-148.

Gu, L., and Knipple, D. C. 2013. Recent advances in RNA interference research in insects: Implications for future insect pest management strategies. Crop Prot. 45:36-40.

Hagen, C., Frizzi, A., Kao, J., Jia, L., Huang, M., Zhang, Y., and Huang, S. 2011. Using small RNA sequences to diagnose, sequence, and investigate the infectivity characteristics of vegetable-infecting viruses. Arch. Virol. 156: 1209-1216.

Hasegawa, D. K., Chen, W., Kaur, N., Wintermantel, W. M., Simmons, A. M., Fei, Z., and Ling, K. 2016. Comparative transcriptome analysis of Bemisia tabaci in response to TYLCV and development of RNA interference to manage whitefly-transmitted viruses. Page 77 in: Proceedings of the 2nd International Whitefly Symposium, 14-19 Feb., 2016, Arusha, Tanzania.

Ho, T., Quito-Avila, D., Keller, K. E., Postman, J. D., Martin, R. R., and Tzanetakis, I. E. 2016. Evidence of sympatric speciation of elderberry carlaviruses. Virus Res. 215:72-75.

Ho, T., and Tzanetakis, I. E. 2014. Development of a virus detection and discovery pipeline using next generation sequencing. Virology 471-473: 54-60.

Honeybee Genome Sequencing Consortium. 2006. Insights into social insects from the genome of the honeybee *Apis mellifera*. Nature 443:931.

Hogenhout, S. A., Ammar, E.-D., Whitfield, A. E., and Redinbaugh, M. G. 2008. Insect vector interactions with persistently transmitted viruses. Annu. Rev. Phytopathol. 46:327-59.

Huang, H. T., Chan, H. L., Shih, T. Y., and Chen, L. L. 2015. A study of the role of glucose transporter 1 (Glut1) in white spot syndrome virus (WSSV) infection. Fish Shellfish Immunol. 46:305-314.

i5K Consortium. 2013. The i5K initiative: Advancing arthropod genomics for knowledge, human health, agriculture, and the environment. J. Hered. 104: 595-600.

International Aphid Genomics Consortium. 2010. Genome Sequence of the pea aphid *Acyrthosiphon pisum.* PLoS Biol. 8:e1000313.

International Committee on Taxonomy of Viruses. 2015. Master Species List, 2015v1. International Committee on Taxonomy of Viruses, Published online. https://talk.ictvonline.org/files/master-species-lists/.

International Whitefly Genome Initiative. 2015. The genome of whitefly Bemisia tabaci, an important plant pest and virus vector. International Whitefly Genome Initiative. Published online. http://www.whiteflygenomics.org

Kaur, N., Chen, W., Zheng, Y., Hasegawa, D. K., Ling, K.-S., Fei, Z., and Wintermantel, W. M. 2016. Transcriptomic studies of *Bemisia tabaci*, the whitefly vector of *Tomato chlorosis virus*. Page 37 in: Proceedings of the 2nd International Whitefly Symposium, 14-19 February 2016. Arusha, Tanzania.

Kollenberg, M., Winter, S., and Gotz, M. 2014. Quantification and localization of *Watermelon chlorotic stunt virus* and *Tomato yellow leaf curl virus* (*Geminiviridae*) in populations of *Bemisia tabaci* (*Hemiptera*, *Aleyrodidae*) with differential virus transmission characteristics. PLoS One 9:e111968.

Koonin, E. V., and Dolja, V. V. 2012. Expanding networks of RNA virus evolution. BMC Biol. 10:54.

Kreuze, J. F., Perez, A., Untiveros, M., Quispe, D., Fuentes, S., Barker, I., and Simon, R. 2009. Complete viral genome sequence and discovery of novel viruses by deep sequencing of small RNAs: A generic method for diagnosis, discovery and sequencing of viruses. Virology 388:1-7.

Kubo, Y., Hayashi, H., Matsuyama, T., Sato, H., and Yamamoto, N. 2012. Retrovirus entry by endocytosis and cathepsin proteases. Adv. Virol. 2012: 640894.

Lapidot, M., and Polston, J. E. 2010. Biology and epidemiology of *Bemisia*-vectored viruses. Pages 227-345 in: *Bemisia*: Bionomics and Management of a Global Pest. Stansley, P. A., and Naranjo, S. E., eds. Springer, Dordrecht.

Leftwich, P. T., Koukidou, M., Rempoulakis, P., Gong, H., Zacharopoulou, A., Fu, G., Chapman, T., Economopoulos, A., Vontas, J., and Alphey, L. 2014. Genetic elimination of field-cage populations of Mediterranean fruit flies. P. Roy. Soc, B-Biol. Sci. 281:1461.

Legg, J. P. 1994. *Bemisia tabaci*: The whitefly vector of cassava mosaic geminiviruses in Africa: An ecological perspective. Afr. Crop Sci. J. 2: 437-448.

Legg, J. P., Sseruwagi, P., Boniface, S., Okao-Okuja, G., Shirima, R., Bigirimana, S., Gashaka, G., Herrmann, H.-W., Jeremiah, S. C., Obiero, H. M., Ndyetabula, I., Tata-Hangy, W., Masembe, C., and Brown, J. K. 2014. Spatio-temporal patterns of genetic change amongst populations of cassava *Bemisia tabaci* whiteflies driving virus pandemics in East and Central Africa. Virus Res. 186:61-75.

Li, R., Gao, S., Berendsen, S., Fei, Z., and Ling, K. S. 2015a. Complete genome sequence of a novel genotype of *Squash mosaic virus*. Genome Announc. 3:e01583-14.

Li, R., Gao, S., Hernandez, A. G., Wechter, W. P., Fei, Z., and Ling, K.-S. 2012. Deep sequencing of small RNAs in tomato for virus and viroid identification and strain differentiation. PLoS One 7:e37127.

Li, R., Zheng, Y., Fei, Z., and Ling, K. S. 2015b. First complete genome sequence of an emerging *Cucumber green mottle mosaic virus* isolate in North America. Genome Announc. 3:300452-15.

Li, R., Zheng, Y., Fei, Z., and Ling, K. S. 2015c. Complete genome sequence of an emerging *Melon necrotic spot virus* isolate infecting greenhouse cucumber in North America. Genome Announc. 3:e00775-15.

Liu, S., Chen, Y., and Bonning, B. C. 2015. RNA virus discovery in insects. Curr. Opin. Insect Sci. 8:54-61.

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.

Martin, B., Collar, J.L., Tjallingii, W., and Fereres, A. 1997. Intracellular ingestion and salivation by aphids may cause the acquisition and inoculation of non-persistently transmitted plant viruses. J. Gen. Virol. 78: 2701-2705.

Maruthi, M. N., Hillocks, R. J., Mtunda, K., Raya, M. D., Muhanna, M., Kiozia, H., Rekha, A. R., Colvin, J., and Thresh, J. M. 2005. Transmission of *Cassava brown streak virus* by *Bemisia tabaci* (Gennadius). J. Phytopathol. 153:307-312.

Morin, S., Ghanim, M., Sobol, I., and Czosnek, H. 2000. The GroEL protein of the whitefly *Bemisia tabaci* interacts with the coat protein of transmissible and nontransmissible begomoviruses in the yeast two-hybrid system. Virology 276:404-416.

Morrison, N. I., Simmons, G. S., Fu, G., Connell, S. O., Walker, A. S., Dafa'alla, T., Walters, M., Claus, J., Tang, G., Jin, L., Marubbi, T., Epton, M. J., Harris, C. L., Staten, R. T., Miller, E., Miller, T. A., and Alphey, L. 2012. Engineered repressible lethality for controlling the pink bollworm, a lepidopteran pest of cotton. PLoS One 7:e50922.

Morse, J. G., and Hoddle, M. S. 2006. Invasion biology of thrips. Annu. Rev. Entomol. 51:67-89.

Nagalakshmi, U., Wang, Z., Waern, K., Shou, C., Raha, D., Gerstein, M., and Snyder, M. 2008. The transcriptional landscape of the yeast genome defined by RNA sequencing. Science 320:1344-1349.

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.

Navarro, B., Pantaleo, V., Gisel, A., Moxon, S., Dalmay, T., Bisztray, G., Di Serio, F., and Burgyán, J. 2009. Deep sequencing of viroid-derived small RNAs from grapevine provides new insights on the role of RNA silencing in plant-viroid interaction. PLoS One 4:e7686.

Navas-Castillo, J., Camero, R., Bueno, M., and Moriones, E. 2000. Severe outbreaks in tomato in Spain associated with infections of *Tomato chlorosis virus*. Plant Dis. 84:835-837.

Nene, V., Wortman, J. R., Lawson, D., Haas, B., Kodira, C., Tu, Z. J., Loftus, B., Xi, Z., Megy, K., Grabherr, M., Ren, Q., Zdobnov, E. M., Lobo, N. F., Campbell, K. S., Brown, S. E., Bonaldo, M. F., Zhu, J., Sinkins, S. P., Hogenkamp, D. G., Amedeo, P., Arensburger, P., Atkinson, P. W., Bidwell, S., Biedler, J., Birney, E., Bruggner, R. V., Costas, J., Coy, M. R., Crabtree, J., Crawford, M., Debruyn, B., Decaprio, D., Eiglmeier, K., Eisenstadt, E., El-Dorry, H., Gelbart, W. M., Gomes, S. L., Hammond, M., Hannick, L. I., Hogan, J. R., Holmes, M. H., Jaffe, D., Johnston, J. S., Kennedy, R. C., Koo, H., Kravitz, S., Kriventseva, E. V., Kulp, D., Labutti,K., Lee, E., Li, S., Lovin, D. D., Mao, C., Mauceli, E., Menck, C. F., Miller, J. R., Montgomery, P., Mori, A., Nascimento, A. L., Naveira, H. F., Nusbaum, C., O'Leary, S., Orvis, J., Pertea, M., Quesneville, H., Reidenbach, K. R., Rogers, Y. H., Roth, C. W., Schneider, J. R., Schatz, M., Shumway, M., Stanke, M., Stinson, E. O., Tubio, J. M., Vanzee, J. P., Verjovski-Almeida, S., Werner, D., White, O., Wyder, S., Zeng, Q., Zhao, Q., Zhao, Y., Hill, C. A., Raikhe, A. S., Soares, M. B., Knudson, D. L., Lee, N. H., Galagan, J., Salzberg, S. L., Paulsen, I. T., Dimopoulos, G., Collins, F. H., Birren, B., Fraser-Liggett, C. M., and Severson, D. W. 2007. Genome sequence of *Aedes aegypti*, a major arbovirus vector. Science 316:1718-1723.

Ng, J. C. K., Tian, T., and Falk, B. W. 2004. Quantitative parameters determining whitefly (*Bemisia tabaci*) transmission of *Lettuce infectious yellows virus* and an engineered defective RNA. J. Gen. Virol. 85:2697-2707.

Ng, T. F. F., Duffy, S., Polston, J. E., Bixby, E., Vallard, G. E., and Breitbart, M. 2011. Exploring the diversity of plant DNA viruses and their satellites using vector-enabled metagenomics on whiteflies. PLoS One 6:e19050.

Nouri, S., Salem, N., Nigg, J., and Falk, B. W. 2016. Diverse array of new viral sequences identified in worldwide populations of the Asian citrus psyllid (*Diaphorina citri*) using viral metagenomics. J. Virol. 90:2434-2445.

Ohnesorge, S., and Bejarano, E. 2009. Begomovirus coat protein interacts with a small heat-shock protein of its transmission vector (*Bemisia tabaci*). Insect Mol. Biol. 18:693-703.

Padmanabhan, C., Gao, S., Li, R., Zhang, S., Fei, Z., and Ling, K. S. 2014. Complete genome sequence of an emerging genotype of tobacco streak virus in the U.S. Genome Announc. 2:e01138-14.

Padmanabhan, C., Zheng, Y., Li, R., Sun, S.-E., Zhang, D., Liu, Y., Fei, Z., and Ling, K. S. 2015. Complete genome sequence of *Southern tomato virus* identified from China using next generation sequencing. Genome Announc. 3:e01226-15.

Phuc, H. K., Andreasen, M. H., Burton, R. S., Vass, C., Epton, M. J., Pape, G., Fu, G., Condon, K. C., Scaife, S., Donnelly, C. A., Coleman, P. G., White-Cooper, H., and Alphey, L. 2007. Late-acting dominant lethal genetic systems and mosquito control. BMC Biol. 5:11.

Pirone, T. P., and Perry, K. L. 2002. Aphids: Non-persistent transmission. Adv. Bot. Res. 36:1-19.

Polston, J. E., DeBarro, P., and Boykin, L. M. 2014. Transmission specificities of plant viruses with the newly identified species of the *Bemisia tabaci* species complex. Pest Manag. Sci. 70:1547-1552.

Pridgeon, J. W., Zhao, L., Becnel, J. J., Strickman, D. A., Clark, G. G., and Linthicum, K. J. 2008. Topically applied *AaeIAP1* double-stranded RNA kills female adults of *Aedes aegypti*. J. Med. Entomol. 45:414-20.

Roossinck, M. J. 2011. The good viruses: Viral mutualistic symbioses. Nat. Rev. Microbiol. 9:99-108.

Roossinck, M. J. 2015. Plants, viruses and the environment: Ecology and mutualism. Virology 479-480:271-277.

Roossinck, M. J., Martin, D. P., and Roumagnac, P. 2015. Plant virus metagenomics: Advances in virus discovery. Phytopathology 105:716-727.

Rosen, R., Kanakala, S., Kliot, A., Pakkianathan, B. C., Farich, B. A., Santana-Magal, N., Elimelech, M., Kontsedalov, S., Lebedev, G., Cilia, M., and Ghanim, M. 2015. Persistent, circulative transmission of begomoviruses by whitefly vectors. Curr. Opin. Virol. 15:1-8.

Rosario, K., Capobianco, H., Ng, T. F. F., Breitbart, M., and Polston, J. E. 2014. RNA viral metagenome of whiteflies leads to the discovery and characterization of a whitefly-transmitted carlavirus in North America. PLoS One 9:e86748.

Sim, S., Ramirez, J. L., and Dimopoulos, G. 2012. Dengue virus infection of the *Aedes aegypti* salivary gland and chemosensory apparatus induces genes that modulate infection and blood-feeding behavior. PLoS Pathog 8: e1002631.

Stafford-Banks, C. A., Rotenberg, D., Johnson, B. R., Whitfield, A. E., and Ullmann, D. E. 2014. Analysis of the salivary gland of transcriptome of *Frankliniella occidentalis*. PLoS One 9:e94447.

Stewart, L. R., Medina, V., Tian, T., Turina, M., Falk, B. W., and Ng, J. C. K. 2010. A mutation in the *Lettuce infectious yellows virus* minor coat protein disrupts whitefly transmission but not *in planta* systemic movement. J. Virol. 84:12165-12173.

Tailor, C.S., Willett, B.J., and Kabat, D. 1999. A putative cell surface receptor for anemia inducing *Feline leukemia virus* subgroup C is a member of a transporter superfamily. J. Virol. 73:6500-6505.

Thomas, D. D., Donnelly, C. A., Wood, R., and Alphey, L. S. 2000. Insect population control using a dominant, repressible, lethal genetic system. Science 287:2474-2476.

Tian, T., Rubio, L., Yeh, H. H., Crawford, B., and Falk, B. W. 1999. *Lettuce infectious yellows virus*: In vitro acquisition analysis using partially purified virions and the whitefly *Bemisia tabaci*. J. Gen. Virol. 80:1111-1117.

Tribolium Genome Sequencing Consortium. 2008. The genome of the model beetle and pest *Tribolium castaneum*. Nature 452:949.

Tzanetakis, I.E., Martin, R.R., and Wintermantel, W.M. 2013. Epidemiology of criniviruses: An emerging problem in world agriculture. Front. Microbiol. 2013:00119. Published online. doi:10.3389/fmicb.2013.00119.

Upadhyay, S. K., Chandrashekar, K., Thakur, N., Verma, P. C., Borgio, J. F., Singh, P. K., and Tuli, R. 2011. RNA interference for the control of whiteflies (*Bemisia tabaci*) by oral route. J. Biosci. 36:153-161.

Walker, B. D., Kowalski, M., Goh, W. C., Kozarsky, K., Krieger, M., Rosen, C., Rohrschneider, L., Haseltine, W. A., and Sodroski, J. 1987. Inhibition of human immunodeficiency virus syncytium formation and virus replication by castanospermine. Proc. Natl. Acad. Sci. USA 84:8120-8124.

Webster, C. L., Waldron, F. M., Robertson, S., Crowson, D., Ferrari, G., Quintana, J. F., Brouqui, J. M., Bayne, E. H., Longdon, B., Buck, A. H., Lazzaro, B. P., Akorli, J., Haddrill, P. R., and Obbard, D. J. 2015. The discovery, distribution, and evolution of viruses associated with *Drosophila melanogaster*. PLoS Biol. 13:e1002210.

Whitfield, A., Falk, B. W., and Rotenberg, D. 2015. Insect vector-mediated transmission of plant viruses. Virology 479-480:278-289.

Whitfield, A. E., Ullman, D. E., and German, T. L. 2005. Tospovirus-thrips interactions. Annu. Rev. Phytopathol. 43:459-89.

Wintermantel, W. M. 2010. Transmission efficiency and epidemiology of criniviruses. Pages 319-331 in: Bemisia: Bionomics and Management of a Global Pest. Stansly, P., and Naranjo, S. E., eds. Springer, New York.

Wintermantel, W. M. 2016. Semipersistent whitefly-transmitted viruses: Criniviruses. Pages 111-119 in: Vector-Mediated Transmission of Plant Pathogens. J. K. Brown, ed. APS Press, St. Paul, MN.

Wintermantel, W. M., Cortez, A. C., Anchieta, A. G., Gulati-Sakhuja, A., and Hladky, L. L. 2008. Co-infection by two criniviruses alters accumulation of each virus in a host-specific manner and influences efficiency of virus transmission. Phytopathology 98:1340-1345.

Wintermantel, W. M., Gilbertson, R. L., McCreight, J. D., and Natwick, E. T. 2016. Host-specific relationship between virus titer and whitefly transmission of *Cucurbit yellow stunting disorder virus*. Plant Dis. 100:92-98.

Wintermantel, W. M., and Wisler, G. C. 2006. Vector specificity, host range, and genetic diversity of *Tomato chlorosis virus*. Plant Dis. 90:814-819.

Wise de Valdez, M.R., Nimmo, D., Betz, J., Gong, H. F., James, A. A., Alphey, L., and Black, W. C., IV. 2011. Genetic elimination of dengue vector mosquitoes. PNAS 108:4772-4775.

Wisler, G. C., and Duffus, J. E. 2001. Transmission properties of whitefly-borne criniviruses and their impact on virus epidemiology. Pages 293-308 in: Virus-Insect-Plant Interactions. K. F. Harris, O. P. Smith, and J. E. Duffus, eds. Academic Press, San Diego, CA.

Wisler, G. C., Duffus, J. E., Liu, H.-Y., and Li, R. H. 1998a. Ecology and epidemiology of whitefly-transmitted closteroviruses. Plant Dis. 82:270-280.

Wisler, G. C., Li, R. H., Liu, H.-Y., Lowry, D. S., and Duffus, J. E. 1998b. Tomato chlorosis virus: A new whitefly-transmitted, phloem limited, bipartite closterovirus of tomato. Phytopathology 88:402-409.

Wu, Q., Ding, S. W., Zhang, Y., and Zhu, S. 2015. Identification of viruses and viroids by Next-generation sequencing and homology-dependent and homology-independent algorithms. Annu. Rev. Phytopathol. 53:425-444.

Wu, Q., Luo, Y., Lu, R., Lau, N., Lai, E. C., Li, W. X., and Ding, S. W. 2010. Virus discovery by deep sequencing and assembly of virus-derived small silencing RNAs. Proc. Natl. Acad. Sci. USA 107:1606-1611.

Wu, Q., Wang, Y., Cao, M., Pantaleo, V., Burgyan, J., Li, W. X., and Ding, S. W. 2012. Homology-independent discovery of replicating pathogenic circular RNAs by deep sequencing and a new computational algorithm. Proc. Natl. Acad. Sci. USA 109:3938-43.

Xia, Q., Zhou, Z., Lu, C., Cheng, D., Dai, F., Li, B., Zhao, P., Zha, X., Cheng, T., Chai, C., Pan, G., Xu, J., Liu, C., Lin, Y., Qian, J., Hou, Y., Wu, Z., Li, G., Pan, M., Li, C., Shen, Y., Lan, X., Yuan, L., Li, T., Xu, H., Yang, G., Wan, Y., Zhu, Y., Yu, M., Shen, W., Wu, D., Xiang, Z., Yu, J., Wang, J., Li, R., Shi, J., Li, H., Li, G., Su, J., Wang, X., Li, G., Zhang, Z., Wu, Q., Li, J., Zhang, Q., Wei, N., Xu, J., Sun, H., Dong, L., Liu, D., Zhao, S., Zhao, X., Meng, Q., Lan, F., Huang, X., Li, Y., Fang, L., Li, C., Li, D., Sun, Y., Zhang, Z., Yang, Z., Huang, Y., Xi, Y., Qi, Q., He, D., Huang, H., Zhang, X., Wang, Z., Li, W., Cao, Y., Yu, Y., Yu, H., Li, J., Ye, J., Chen, H., Zhou, Y., Liu, B., Wang, J., Ye, J., Ji, H., Li, S., Ni, P., Zhang, J., Zhang, Y., Zheng, H., Mao, B., Wang, W., Ye, C., Li, S., Wang, J., Wong, G. K., and Yang, H. 2004. A draft sequence for the genome of the domesticated silkworm (*Bombyx mori*). Science 306:1937-1940.

Xu, J., Liu, S. S., and DeBarro, P. 2010. Reproductive incompatibility among genetic groups of *Bemisia tabaci* supports the proposition that the whitefly is a cryptic species complex. Bull. Entomol. Res. 100:359-366.

Zhang, H., Li, H., Miao, X. 2013a. Feasibility, limitation and possible solutions of RNAi-based 18 technology for insect pest control. Insect Sci. 20: 15-30.

Zhang Z, Zhang P, Li W, Zhang J, Huang F, Yang J, Bei Y, and Lu Y. 2013b. De novo transcriptome sequencing in *Frankliniella occidentalis* to identify genes involved in plant virus transmission and insecticide resistance. Genomics 101:296-305.

Zotti, M., and Smagghe, G. 2015. RNAi technology for insect management and protection of beneficial insects from diseases: Lessons, challenges and risk assessments. Neotrop. Entomol. 44:197-213.