March 2019

Effects of Plant Viruses on Vectors and Non-vector Herbivores in Three Different Pathosystems

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EFFECTS OF PLANT VIRUSES ON VECTORS AND NON-VECTOR HERBIVORES IN THREE DIFFERENT PATHOSYSTEMS

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and Agricultural
and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Entomology

by
Sunil Paudel
B. S., Tribhuvan University, 2008
M.S., University of Idaho, 2013
May 2019

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my major advisor, Dr. Jeffrey A. Davis for his continuous support throughout my graduate studies, for his patience, encouragement, and guidance that helped me to grow as a researcher and a critical thinker. I am also thankful to my dissertation committee members, Drs. Michael J. Stout, Fangneng Huang, Jeffrey W. Hoy, and Dean's representative Dr. John C. Larkin for serving on my committee and providing me valuable suggestions and guidance for improvement.

My sincere thanks go to Art Richter, Mark Murray, Monique de Souza, John Dryburgh, Colin Bonser, Scot Lee and all the members of soybean entomology laboratory for their help in carrying out my research projects and their friendship throughout this journey.

I am always grateful to the unconditional support of my family members for this academic accomplishment. I owe special thanks to my wife, Shilpa Ghimire, for her love, support, understanding and patience throughout this journey.

Last but not the least, my sincere thanks go to the Department of Entomology, all the faculty members, and all my friends for their friendship and company during my graduate studies here at Louisiana State University.

TABLE OF CONTENTS

ABSTRACT

Plant viruses are an important component of agro-ecosystems and the knowledge of impacts they can cause on their hosts, and on different vectors and non-vector herbivores associated with the hosts, is very crucial in devising sound management strategies for virus disease and vector control in the landscape. The interactions between these components, however, are difficult to predict and vary according to the system under investigation. In order to understand some of these interrelationships, different sets of experiments were carried out in three different pathosystems to look at the impacts of plant viruses on vector and non-vector herbivores. Firstly, in *Sorghum mosaic virus* (SrMV)-sorghum (*Sorghum bicolor* L.) pathosystem, we found that *Melanaphis sacchari* (Zehntner) was attracted to virus infected sorghum but the population was negatively affected upon feeding on virus infected sorghum. Moreover, *M. sacchari* unlike *Myzus persicae* (Sulzer), failed to transmit SrMV, and we state based on our results that *M. sacchari* is a non-vector of SrMV. In *Cucumber mosaic virus* (CMV) and *Sunn-hemp mosaic virus* (ShMV) infected cowpea (*Vigna unguiculata* (L.) Walp), oviposition by adult *Chrysodeixis includens* (Walker) and *Spodoptera frugiperda* (Smith) was negatively affected whereas *S. frugiperda* larva benefitted upon feeding on CMV-infected host tissue. In our study with *Bell pepper endornavirus* (BPEV) in bell pepper (*Capsicum annuum* L.), we observed that *M. persicae* preferred virus-free leaves and performed poorly on virus infected leaf tissues. The mixed results we obtained on the impacts of plant viruses in different systems suggest that it is difficult to draw a general conclusion and the interactions are complex, diverse, and virus-insect specific.

CHAPTER 1: GENERAL INTRODUCTION

Plant viruses are abundant in both natural and man-made ecosystems and upon infecting alter the physiological as well as the phenotypic characteristics of their host plants (Blua et al. 1994, Jeger et al. 2004, Agrawal et al. 2006). Moreover, plant viruses are obligate intracellular parasites that lack their own molecular machinery to replicate and therefore need to infect a plant to do so. Since their hosts are sessile, plant viruses need to employ different strategies to disperse within the landscape. The majority of plant viruses recruits biotic vectors for transmission to cause disease in agricultural crops (Whitfield et al. 2015). One of these biotic agents are insects, the largest group of plant virus vectors, that include aphids, thrips, leafhoppers, plant hoppers and whiteflies (Bragard et al. 2013).

Along with the changes plant viruses cause to their hosts, they also influence vector behavior (Eigenbrode et al. 2002, Ingwell et al. 2012) and population dynamics (Ajayi et al. 1983, Alvarez et al. 2007). The effects of plant viruses on their vectors, however, are not predictable and depend upon factors such as the type of virus or mode of transmission. Aphid vectors performed better on plants infected by persistently transmitted viruses (Araya and Foster 1987, Montllor and Gildow 1986, Fereres et al. 1989, Castle and Berger 1993, Jimenez-Martinez et al. 2004), whereas reduced performance of aphids was reported on the plants infected by non-persistently transmitted virus (Mauck et al. 2010).

Although information abounds on the impacts of plant viruses on vector herbivores, plant virus and non-vector herbivore interactions have been poorly illustrated. Understanding these interactions is critical for developing pest management strategies in diverse and complex agro-ecosystems since most insect herbivores do not serve as vectors under field conditions (Hu et al. 2013). Only a few studies have documented the effects of plant viruses on non-vector herbivores

(Hare and Dodds 1987, Lin et al. 2008, Thaler et al. 2010, Mauck et al. 2010). Therefore, we sought to study the interactions between plant viruses, their potential vectors and non-vector herbivores on three distinct pathosystems with an aim to understand these complex interrelationships.

The first two data chapters of this dissertation (Chapter 3 and 4) focusses on *Sorghum mosaic virus* (SrMV) -grain sorghum (*Sorghum bicolor* L.) pathosystem. *Sorghum mosaic virus* (SrMV) (Family: Potyviridae, Genus: Potyvirus) is an important potyvirus that causes mosaic disease in sugarcane *Saccharum officinarum* L.), maize (*Zea mays* L.), sorghum, and other *Poaceae* species (Grisham et al. 2007, Xu et al. 2008). SrMV causes a reduction in grain and forage production in susceptible sorghum cultivars (Silva et al. 2012). Even though SrMV is more prevalent in sugarcane than sorghum in field conditions in Louisiana, there is a possibility of a host switch or expansion, like in the cases of other viruses historically related to sugarcane (Wei et al. 2016, de Souza 2017). Wei et al. (2016) reported that *Sugarcane yellow leaf virus* (SCYLV) (Luteoviridae: *Polerovirus*), a disease historically related with sugarcane, was detected in 41% of sorghum plants tested in Florida. Similarly, de Souza (2017) reported the incidence of JGMV (*Johnson grass mosaic virus*) infecting sorghum in Brazil. One of the potential vectors of SrMV, we presume, is *Melanaphis sacchari*, an important invasive pest in U.S. sorghum. *M. sacchari* was reported to transmit *Sugarcane mosaic virus* (SCMV) (Family: Potyviridae, Genus: Potyvirus) in sorghum (Singh et al. 2005). We hypothesized a similar mode of transmission of SrMV by *M. sacchari* in sorghum. Outbreaks of *M. sacchari* have caused tremendous economic loss as a direct pest to sorghum and hence its potential to vector plant viruses adds a new dimension to the injury it can inflict in crop production systems. Therefore, we designed experiments to study the potential of *M. sacchari* to vector and transmit SrMV in sorghum. We

compared the efficiency of *M. sacchari* to vector SrMV with a model vector of potyviruses, *Myzus persicae* (Sulzer). Additionally, we conducted electrical penetration graph (EPG) studies and characterized the feeding behavior of *M. persicae* and *M. sacchari* in order to determine if the differences in their feeding behaviors could correlate with their respective transmission efficiencies. We also designed experiments to understand the preference behavior of *M. persicae* and *M. sacchari* on SrMV-infected and non-infected sorghum. The life history traits of *M. sacchari* on SrMV-infected and non-infected sorghum was also studied.

Chapter 5 of this dissertation documents the study conducted on *Cucumber mosaic virus* (CMV) and *Sunn-hemp mosaic virus* (ShMV) infected cowpea (*Vigna Unguiculata* (L.) Walp) and the effects on two non-vector herbivores, soybean looper, *Chrysodeixis includens* (Walker) and fall armyworm, *Spodoptera frugiperda* (Smith). Both CMV and ShMV are important viruses that infect cowpea and cause mosaic symptoms, stunted growth, and eventual yield loss (Arogundade et al. 2009, Pio-Ribeiro et al. 1978). Unlike the earlier chapters which studied piercing-sucking herbivores and impacts of plant viruses on them in a relatively understudied pathosystem, here, we studied two economically important chewing herbivores in two well-defined pathosystems, CMV and ShMV in cowpea. The effects of CMV and ShMV-infected cowpea on soybean looper and fall armyworm larval growth and adult oviposition preference was investigated.

The third pathosystem studied in this dissertation (Chapter 6) consists of a persistent virus, *Bell pepper endornavirus* (BPEV) in bell pepper (*Capsicum annuum* L.) and a common piercing sucking herbivore, green peach aphid. This is a unique pathosystem which includes a plant virus with no documented symptoms and no negative effects on the hosts which is present in almost all tested bell pepper cultivars (Valverde and Fontenot 1991). There is no known vector

of BPEV. It is transmitted only vertically and is present at uniform concentrations in every tissue and at every developmental stage of the plants (Okada et al. 2011). BPEV-infected genotypes seemed to have been selected and introduced inadvertently in bell pepper growing regions. This selection for virus infected genotypes suggests that viruses might be providing a benefit to the hosts. The benefits might include tolerance or resistance to biotic and abiotic agents and therefore a symbiotic relationship might have evolved between the virus and their host. Escalante (2017) reported that BPEV-infected lines of bell pepper were less severely affected by *Pepper mild mottle virus* (PMMoV) infection. Therefore, in order to understand if BPEV-infection provides any benefit to bell pepper hosts against a common pest, green peach aphid, we designed experiments to determine its host suitability and population dynamics on BPEV-infected and non-infected pepper plants.

As a synopsis, the overarching goal of the studies documented in this dissertation is to understand the effects of plant viruses on the behavior and performance of their potential vectors and non-vector herbivores.

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CHAPTER 2: LITERATURE REVIEW

2.1. Biology and life cycle of aphids

Aphids are insects in the Superfamily Aphidoidea, Family Aphididae, in the Suborder Sternorrhyncha and the homopterous division of the Order Hemiptera. Aphids are small (1-10 mm), soft bodied, and ovoid, with long antennae, piercing-sucking mouth parts, and a pair of cornicles (tubes pointing upward on the dorsal fifth abdominal segment), which secrete alarm pheromones (Brisson and Stern 2006). Distributed worldwide and with more than 4000 known species, aphids include many of the most important insect pests of agriculture in temperate regions (Minks and Harrewijn 1989, Dixon 1998). Aphids can be strictly monophagous, e.g. the grape phylloxera, *Daktulosphara vitifoliae* Fitch, to polyphagous, e.g. the green peach aphid, *Myzus persicae* Sulzer (Dixon 1987). Aphids display polymorphism and can possess either apterous (wingless) or alate (winged) forms depending on their function and environmental conditions (Blackman and Eastop 2000, Williams and Dixon 2007).

There are two major types of life cycle of aphids depending upon how they utilize their host plants; most are autoecious or monoecious, living on one or a few species of plants of a particular genus (Eastop 1973), but about 10% are heteroecious, alternating between a primary host and one or more secondary hosts (Dixon 1987). Heteroecious aphid species live on one plant species during winter, known as a primary host, and migrate to an unrelated secondary host during summer (secondary host), migrating back to the primary host in autumn (Williams and Dixon 2007). Depending upon the mode of reproduction, the life cycle of aphids can be categorized as anholocyclic, in which all reproduction is parthenogenetic, or holocyclic, in which a sexually reproducing generation occurs at least once in a year (Dixon 1987). For heteroecious, holocyclic species, sexual morphs (androparae: sexual males that mate with sexual females and

oviparae: sexual females that oviposit eggs after mating with sexual males) are produced on the secondary host while mating occurs in association with the primary host where females oviposit (Miyazaki 1987). The eggs are usually cold tolerant (can tolerate temperatures up to -40 °C), overwinter on the primary host, and hatch in spring, giving rise to fundatrices (James and Luff 1982, Williams and Dixon 2007). For example, *M. persicae* alternates between its primary hosts, usually *Prunus sps*. (Rosaceae) and secondary hosts, various herbaceous plants, in the areas of cold winters, thus displaying a heteroecious, holocyclic life cycle (Blackman 1974). The fundatrix is the most fecund aphid morph and produces numerous offspring through thelytoky (parthenogenesis that produce only females). Spring migrants then leave the primary host and move to secondary hosts, usually an abundant herbaceous plant where they produce numerous parthenogenetic generations and build a large population during summer (Williams and Dixon 2007, Dixon 2012). Migration of heteroecious aphid species back to the primary host from secondary hosts is brought about by shortened day length and fall in temperature (Williams and Dixon 2007). Some aphid species, however, also exhibit plasticity in life cycles depending upon the climate or the availability of primary hosts. For example, *M. persicae* is anholocyclic in more temperate regions of its range while some *Diuraphis noxia*, though holocyclic in its native range, displays anholocyclic life cycles in its invaded range, North America (Williams and Dixon 2007). Aphid species, if anholocyclic, do not produce sexual morphs but rather overwinter as parthenogenetic nymphs or adults (Williams and Dixon 2007, Dixon 2012).

Aphids feed passively on vascular tissue contents (generally the phloem) via high pressure within the sieve elements. Their mouthparts consist of maxillae and mandibles elongated into a stylet bundle that penetrates plant tissues and reach the phloem, while the distal tip of the labium helps to guide stylet penetration from outside. Aphids are known to produce

two different types of saliva during feeding that mediate their interactions with plants (Miles 1999). The first type, known as gel saliva, is dense and proteinaceous and is secreted during stylet movement through the apoplast. It jells around the stylets, forming a stylet sheath to facilitate penetration, seal puncture sites on cell membranes, isolate plant tissues from mouth parts, and prevent plant reaction at the site of feeding (Felton and Eichenseer 1999). The second type is watery saliva which is secreted during cell penetration and ingestion. Once the stylets have reached the phloem, digestive and lytic enzymes are released and aphids start sucking plant photosynthates, leading to plant injury (Miles 1999).

Injury by aphids can be categorized into direct and indirect injury. Direct injury occurs due to the removal of plant photosynthates and piercing of the plant foliage leading to deformation, necrosis, or stunting of the leaves (Breen and Teetes 1990, Kindler and Hammon 1996, Blackman and Eastop 2000). This type of feeding often causes weakening and yellowing of the plant (Burd 1993, Blackman and Eastop 2000). Indirect injury is associated with sooty mold growth which leads to reduced photosynthetic ability or by vectoring of plant viruses (Quisenberry and Ni 2007).

Aphids feed on plant sap which is a nutritionally unbalanced diet high in carbon. This leads to nitrogen deficiency in aphids. Aphids are known to possess the ability to synthesize amino acids and lipids from dietary sugars (Febvay et al. 1999). Small organic compounds like sugars and amino acids are principal components in phloem sap which require minimum digestive processing and increase assimilation efficiency of aphids. However, plant sap doesn't provide all the required essential amino acids. In order to meet the requirements aphids lack in their diet, aphids have developed an obligatory symbiotic relationship with a gamma-proteobacteria, *Buchnera aphidicola* Munson Baumann and Kinsey, hosted in the specialized

cells (mycetocytes or bacteriocytes) in the cytoplasm (Munson et al. 1991). These endosymbionts secrete essential amino acids to supplement the aphid diet (Douglas and Prosser 1992, Douglas 1998, Douglas et al. 2001).

Aphids possess a differentiated digestive system that includes a filter chamber, a structure that links the initial portion of the foregut (stomodeum) to the anterior portion of the hindgut (proctodeum). Incoming plant sap has a higher osmotic pressure than the hemolymph of aphids due to the higher concentration of dietary sugars (Douglas 2006). In order to prevent desiccation, the ingested sap is allowed to pass quickly and directly to the final portion of the digestive system, thereby circumventing the main absorptive portion of the midgut (Waterhouse 1957, Douglas 2006).

2.2. Aphid transmitted viruses, their biology, and management

Plant viruses must move from infected to healthy plants in order to survive and spread. Since they cannot move by themselves, plant viruses achieve this either by mechanical means or by exploiting biological vectors such as arthropods, nematodes, and fungi. Of all known plant virus vectors, the best described insect vectors are aphids, thrips, leafhoppers, plant hoppers, and whiteflies (Bragard et al. 2013). More than 190 aphid species out of 4,700 described species have been reported to transmit plant viruses (Remaudiére and Remaudiére 1997, Nault 1997). Most common vector species belong to genera *Myzus*, *Acyrthosiphon*, *Aphis,* and *Macrosiphum* (Kennedy et al. 1962). Plant viruses transmitted by aphids belong to about 19 virus genera comprising around 275 virus species, many of which cause diseases of major economic importance in agricultural crops (Nault 1997).

Virus transmission by aphids typically consists of four stages: i) acquisition- the process by which aphids acquire virus particles from the infected host upon feeding on them; ii)

retention- process by which virus particles are retained at specific sites within or on the vector; iii) latency- duration required by the virus particle to be infective after acquisition before which transmission cannot occur; and iv) inoculation- the process by which virus particles are released into the tissues of a healthy plant by the vector (Katis et al. 2007). Based on the time required for these four stages, three modes of transmission have been initially delineated; non-persistent, semi-persistent, and persistent transmission. Moreover, depending on whether the ingested virus particles circulate inside the body of vector before transmission or not, the mode of virus transmission has been broadly categorized into circulative and non-circulative transmission (Ng and Perry 2004).

Non-circulative transmission includes both non-persistent and semi-persistent modes of transmission. The majority of the aphid-vectored plant viruses are transmitted in a non-circulative manner (Ng and Perry 2004, Fereres and Moreno 2009). Viruses transmitted in a non-circulative fashion are lost upon molting by aphids. Non-persistent transmission is characterized by very short acquisition periods of a few seconds to minutes, retention period of few minutes to hours, and no latent period (Ng and Falk 2006). Also known as stylet-borne viruses because these viruses are retained in the insect stylet, non-persistently transmitted viruses only occur in aphids, not in other virus vectors (Ng and Falk 2006). Some examples of non-persistently transmitted viruses are *potyvirus (*type species: *Potato virus Y*)*, carlavirus* (type species: *Carnation latent virus*)*, cucumovirus* (type species: *Cucumber mosaic virus*)*, bromovirus* (type species: *Brome mosaic virus*)*, caulimovirus* (type species: *Cauliflower mosaic virus*) (Nault 1997, ICTV 2017). Non-persistent plant viruses have been suggested to employ one of two mechanisms of transmission: capsid-only or helper-dependent (Ng and Perry 2004, Ng and Falk 2006, Bragard et al. 2013, Blanc et al. 2014). For example, *Cucumber mosaic virus* (CMV)

particles were shown to require only viral coat protein and conserved capsid surface domains for efficient transmission by *M. persicae* (Liu et al. 2002). Potyviruses encode a helper protein, helper component-proteinase (HC-Pro), which is made in the plant and has to be acquired by the aphid either during or before virus acquisition for transmission to occur (Raccah et al. 2001). HC-Pro facilitates virion retention in aphid stylets by acting as a bridge between potyvirus CP (capsid protein) and aphid proteins in the stylet (Blanc et al. 1998, Dombrovsky et al. 2007, Seo et al. 2010). Non-persistent viruses are found to be non-tissue specific (Hogenhout et al. 2008).

In semi-persistent transmission, acquisition of virus particles by the vectors occurs within minutes and particles are typically ingested in the foregut. These viruses are also known as foregut borne viruses. Acquisition periods for semi-persistent viruses' range from minutes to hours, retention time from few hours to days, and latent periods are absent like that of non-persistently transmitted viruses. Palacios et al. (2002) suggested that prolonged feeding by a vector might increase the transmission efficiency of these viruses. Although most of these viruses are foregut-borne, *Cauliflower mosaic virus*, was found to reside exclusively at the tip of aphid stylet (Uzest et al. 2007). Semi-persistent viruses are not found to be internalized in the vector gut but rather reside in chitin-lined areas of the foregut (Ng and Falk 2006). Viral CP is largely involved in virus acquisition and retention by the insect vector in semi persistent transmission (Ng and Zhou 2015). Some common examples of semi-persistently transmitted viruses are *closterovirus* (type species: *Beet yellows virus*), *caulimovirus* (type species: *Cauliflower mosaic virus*) (in part), *crinivirus* (type species: *Lettuce infectious yellows virus*) (Nault 1997, Hull 2002, ICTV 2017).

Persistently transmitted viruses are almost always circulative, in which the ability to transmit virus is retained between molts. Circulative, persistent viruses require longer acquisition

periods than non-persistent viruses, lasting minutes to hours with retention times being the entire life of the insect and latent periods lasting days to weeks (Ng and Falk 2006). A latent period occurs between acquisition and inoculation, but once it has passed, the aphid can remain infective for life. Due to the phloem limited nature and the path they have to travel inside the host, these viruses have a longer acquisition and retention periods (Ng and Perry 2004). Transmission of circulative, persistent viruses from an infected plant to a healthy host plant through aphid vectors have been described to consist of four distinct processes: (1) ingestion/intake of the virus from infected plant to aphid's alimentary canal lumen, (2) virus acquisition through aphid's gut [Poleroviruses like *Beet western yellows virus* and *Potato leafroll virus* use posterior mid gut of *M. persicae* (Garret et al. 1993, Reinbold et al. 2001)) while viruses like *Barley yellow dwarf virus* in *Sitobion avenae*, *Cereal yellow dwarf virus*, *Soybean dwarf virus* in *Aulacorthum solani* and *M. persicae* are found to be internalized at hindgut (Gildow et al. 1994, Brault et al. 2005)], (3) virus retention in the tissues and hemocoel, and (4) transmission of virus particles through accessory salivary gland and into the phloem tissue of a new host plant (Gray and Gildow 2003). Virus particles have to overcome distinct barriers in order to circulate and move across the tissue of vector and this movement is brought about by the process of membrane mediated endocytosis and exocytosis. Different barriers to movement for virus particles across the body of vector for persistent, circulative transmission involve: a) midgut infection barrier, b) dissemination barrier, c) salivary glands escape barrier, and d) transovarial transmission barrier (Ammar 1994). The existence of these barriers accounts for the specificity of transmission of these viruses by different aphid species (Peiffer et al. 1997).

Based on the ability of the virus to replicate in vector cells, circulative viruses are further categorized as propagative (eg. rhabdoviruses) or non-propagative (eg. luteoviruses, nanoviruses,

enamoviruses, poleroviruses). Circulative propagative viruses are capable of inducing disease in the vector but rarely do so because they multiply at a very low rate unlike in their host plant where they multiply exponentially, causing high levels of damage in a short period of time (Brault et al. 2010). Plant rhabdoviruses are retained by the infected insect for life and the virus can also follow a transovarial route to reach the offspring (Redinbaugh et al. 2005).

Insect vectors play an important role in deciding the host range of a plant virus. Distinct molecular interactions of virus and vector, genetic components of the vector, and host plant components ingested during feeding determine vector competency (Dietzgen 2016). Thus, controlling aphid vectors is a crucial management strategy for aphid-borne virus diseases in plants. Exploitation of resistance genes of the host to virus and/or vector in order to interfere with virus transmission, chemical control of the vectors, and integrated pest management are some of the most widely used strategies for controlling vector-borne virus diseases (Whitfield et al. 2015, Dietzgen 2016). Potential approaches to disrupt insect transmission of plant viruses from virus acquisition by the vector and transport and delivery to a new host have been discussed in a review by Whitfield and Rotenberg (2015). Cultural control practices to minimize hosts for pathogens such as pruning, rogueing, and removal of volunteer crop plants can prevent uptake of pathogens by the potential vector. Use of reflective mulches and paints to repel vectors, protective row covers and barrier crops can help protect susceptible host plants from virus transmission by vectors (Halbert 2008). Though efforts to introduce natural enemies of virus vectors are underway, it has not been very successful at present (Halbert 2008). Regulatory measures like quarantines, strict sanitation measures in the field, and crop-free periods can also help to control vectored plant pathogens. Chemical control of vectors is an important strategy to limit spread of vectored plant pathogens (Broadbent 1957).

However, the effectiveness of insecticides in managing aphid vectored viruses greatly depends on the mode of transmission. Persistently and semi-persistently transmitted viruses require longer times for acquisition and have a longer latent period, which makes their management by insecticides more feasible than non-persistently transmitted viruses. Insecticides can be used to reduce vector populations or alter their behavior and thereby reduce virus transmission (Broadbent et al. 1956, Heinrich 1979, Perring et al. 1999). However, non-persistently transmitted viruses are difficult to manage with insecticides because of their very short acquisition and inoculation periods that last for very few seconds (Broadbent et al. 1956, Perring et al. 1999). In addition, there have also been reports that insecticides cause irritation and induce greater vector activity which further exacerbates the spread of viruses (Shanks and Chapman 1965, Budnik et al. 1996). Non-colonizing aphids can also be important non-persistent virus vectors as these viruses are transmitted during brief, initial probes in the process of host selection (Perring et al. 1999). Hence, it is very difficult to expose these non-colonizing vectors to a lethal dose of insecticide to kill them rapidly and reduce spread (Broadbent 1969, Loebenstein et al. 1980, Maelzer 1986).

The use of resistant cultivars has been effective to some extent to control viral diseases caused by non-persistently transmitted viruses which are difficult to control by other means (Walker et al. 1982, Hammond 1998). Many commercial crops have been bred for resistance to various viruses such as *Papaya ringspot virus* and *Cucurbit mosaic virus* in melons and potato viruses in potatoes (Berger and German 2001, Brown and Corsini 2001). Mineral and vegetable oils might inhibit non-persistent vector transmission of viruses by modifying the vector probing behavior responsible for transmission (Chavan 2015). The physico- and electrochemical properties of oils might affect insect feeding apparatus and inhibit sap sampling (Simmons et al.

1977). Different modalities of host plant resistance like antixenosis, antibiosis, and tolerance are being employed in resistance breeding against aphid vectors but these approaches are only suitable for persistent viruses, transmitted by colonizing aphids (Katis et al. 2007). Moreover, host-plant resistance tactic is further hindered by the rapid evolution of aphid populations, commonly referred as biotypes, which have overcome host-plant resistance (Katis et al. 2007) Biotype can be defined as "populations within an arthropod species that differ in their ability to utilize a particular trait in a particular plant genotype" (Smith 2005).

2.3. Why aphids are efficient virus vectors?

Aphids are far and away the most widespread and efficient vectors of plant viruses (Nault 1997). Certain characteristics of aphids during host selection process such as series of brief probes, minimum injury to plant tissues during feeding, and rapid dispersal in the event of host rejection predispose them to being very effective virus vectors (Brault et al. 2010). The host range of aphids and their life cycle characteristics such as autoecious or heteroecious, alate or apterae, colonizing or non-colonizing are also key components in determining the virus epidemiology and rate of spread of viruses (Katis et al. 2017). Migrant aphids primarily locate plant materials through visual cues especially color (Kennedy et al. 1959, Kring 1972). After alighting on plants, host recognition process initiates with the help of certain physical and chemical stimuli that are evaluated by various sensilla located on the head, antennae, tarsi and labium of aphids (Pettersson 1971, Tjallingii 1978, Backus 1988, Park and Hardie 2004). Aphids, upon tarsal contact with any solid surface, make brief probes (stylet insertions) as a behavioral reflex, regardless of whether the plant is a host or non-host species and even if there is the presence of deterrent cues (Powell et al. 1999). This behavior of aphids to initiate probing upon landing on any solid surface helps to explain their exceptional ability to transmit plant viruses. Moreover,

probing behavior is essential for aphids to assess internal plant chemistry during host plant selection process (Powell and Hardie 2000, Powell et al. 2006). Virions of non-persistent viruses are acquired and inoculated during these brief epidermal cell punctures (probes) (Powell 1991, Powell et al. 1992), which explains why non-persistent transmission is virtually exclusive to aphids. Aphid stylets lack chemoreceptors and hence aphids need to ingest plant sap through the maxillary food canal to allow chemosensory assessment by a gustatory organ in the pharyngeal area of the foregut (Wensler and Filshie 1969) and during this process, virus particles are ingested and acquired by aphids (Powell et al. 1995, Pirone and Perry 2002). This property of reflex probing and feeding behavior make aphids ideal agents to carry virus particles and rapidly spread them in the ecosystem.

2.4. Effects of plant viruses on behavior and performance of vectors and non-vector herbivores

Since most plant viruses are transmitted by insect vectors, their spread from plant to plant depends on the behavior and dispersal of their vectors (Ng and Falk 2006, Fereres and Moreno 2009, Hogenhout 2008). Thus, plant viruses evolve mechanisms to modify the behavior of their vectors by inducing changes in the plant host in order to enhance virus transmission and spread. The effects of viruses on their vectors can be both indirect or plant mediated, or direct which occurs within the vectors after virus acquisition such as alterations in vector behavior, performance, or both, with implication for the spread and persistence of the pathogen in the host populations (Hurd 2003, Lefevre and Thomas 2008, Rajabaskar et al. 2014, Eigenbrode et al. 2018).

There have been numerous instances of virus-vector-host interactions leading to positive, negative, and neutral effects on vectors. Some persistently transmitted plant viruses have been demonstrated to induce changes in the host plants that cause vectors to preferentially settle on

infected plants, with the potential for increased virus spread (Eigenbrode et al. 2002, Srinivasan et al. 2006, Alvarez et al. 2007, Mauck et al. 2018). Alvarez et al. reported that *Potato leafroll virus* (PLRV) infected mature leaves of potato attracted more *M. persicae* than non-infected leaves. Moreover, it has also been shown that feeding preferences of insect vectors, after the acquisition of a virus, especially for non-persistently transmitted viruses, can be altered (Stafford et al. 2011, Ingwell et al. 2012, Rajabaskar et al. 2014). Rajabaskar et al. (2014) in their study on the potato-*Myzus persicae- Potato leafroll virus* pathosystem, found out that non-viruliferous aphids (aphids not carrying viruses) preferred plants infected with PLRV, whereas viruliferous aphids (aphids carrying viruses) preferred plants not infected with PLRV. Among non-persistently transmitted virus pathosystems, vectors have been found to primarily settle on infected plants initially in some instances (Mauck et al. 2010, Mauck et al. 2014, Carmo-Sousa et al. 2016) while preference for non-infected plants by vectors have also been reported in some other pathosystems (Blua and Perring 1992a). Additionally, some neutral preference behavior by vectors have also been observed in some other non-persistently transmitted virus pathosystems (Blua and Perring 1992b, Castle et al. 1998, Hodge and Powell 2008, Boquel et al. 2012). It is notable to mention that same aphid species can react differently to virus infected plants depending upon the mode of virus transmission (Fereres and Moreno 2008).

In addition to altering vector preference and feeding behavior, virus induced changes in host plants affect the performance of arthropod vectors both positively (Araya and Foster 1987, Fereres et al. 1989, Jimenez-Martinez et al. 2004, Maris et al. 2004, Srinivasan et al. 2008) and negatively (Blua and Perring 1992, Garcia et al. 2000, Stumpf and Kennedy 2005). However, no effects of virus induced host changes on vector performance have been reported in some other instances (Wijkamp et al. 1995, Roca et al. 1997). Additionally, the results of those studies

suggest that there is an interesting pattern of variation on performance of vectors depending upon the mode of transmission. Performance of aphid vectors have been documented to increase on plants infected by persistently transmitted viruses (Araya and Foster 1987, Montllor and Gildow 1986, Fereres et al. 1989, Castle and Berger 1993, Jimenez-Martinez et al. 2004), whereas reduced performance of aphids was reported on the plants infected by non-persistently transmitted virus (Mauck et al. 2010). Fereres et al. 1989 reported that *Sitobion avenae* (F.) had a shorter developmental time, greater fecundity and greater intrinsic rate of increase while feeding on *Barley yellow dwarf virus* (a persistently transmitted virus) infected wheat as compared to aphids feeding on non-infected plants. However, Wosula et al. (2013) reported that mixed infection of sweet potato cultivar Evangeline (*Ipomoea batatas* L.) with three potyviruses; *Sweet potato feathery mottle virus* (SPGMV), *Sweet potato virus 2* (SPV2) *and Sweet potato virus G* (SPVG) positively affected the intrinsic rate of increase and the net reproductive rate of *Myzus persicae*. In contrast, Mauck et al. (2010) found that CMV-infected squash reduced population growth of its two aphid vectors; *Aphis gossypii* and *Myzus persicae*.

Although there is a copious amount of literature on the impacts of plant viruses on vector herbivores, the interactions between plant viruses and non-vector herbivores have been poorly documented. Increment in the growth of *Leptinotarsa decemlineata* (Say) larvae on *Tobacco mosaic virus* (TMV) infected tomato (*Solanum lycopersicum* L.) was reported by Hare and Dodds (1987). Similarly, the larval growth of *Spodoptera exigua* (Hubner) was higher on TMV-infected tomato plants as compared to control plants (Thaler et al. 2010). However, no effects on the feeding or oviposition preference of corn earworm *Helicoverpa armigera* (Hubner) on tomato plants infected by *Tomato mosaic virus* was reported by Lin et al. (2008). In one study, Mauck et al. (2010) reported that *Anasa tristis* (DeGeer), preferred to lay more eggs on healthy

squash (Cucurbita pepo L.) plants as compared to CMV infected plants. Beillure et al. (2010) found that spider mite (*Tetranychus urticae* Koch) survival and oviposition was enhanced by *Tomato spotted wilt virus* (TSWV) infection on pepper plants (*Capsicum annuum* L.). However, fungus gnat (*Bradysia* sp.) females preferred to oviposit on non-infected rather than *White clover mosaic virus* infected white clover (*Trifolium repens* L.) plants, although fungus gnat larvae were only marginally affected by the virus (van Molken et al. 2012). Therefore, the effects of virus infection of the host plant on the behavior and fitness of non-vector herbivores is, at present, unpredictable. It is evident that most insect herbivores under field conditions do not serve as vectors and hence, it is important to understand the effects of virus-infected plants on non-vectors in order to devise efficient pest management tactics in the agro-ecosystems (Hu et al. 2013).

2.5. Use of electrical penetration graph for study of vector feeding behavior

Understanding the feeding behavior of vectors is very important in order to elucidate virus transmission mechanism. Aphids, like any other piercing sucking insects, conduct plant recognition and acceptance behavior internally during stylet penetration in plant tissues (Powell et al. 2006). However, stylet penetration behavior cannot be observed directly but can be monitored using electrical penetration graph (EPG) technique (McLean and Kinsey 1964). EPG is an innovative approach which is useful in assessing stylet probing behavior of plant feeding insects, usually hemipterans such as aphids (McLean and Kinsey 1964). It is a simple system which consists of a host plant and a piercing-sucking insect attached through a wire in an electrical circuit, which is completed as soon as the mouthparts of the insect penetrates the plant. Penetration activities generate electrical events inside the stylet canals or at its extremities (Tjallingii, 1988). Two different systems can be used, the AC system (McLean and Kinsey,

1964) and the DC system (Tjallingii 1988) and both the systems record the changes in electrical resistance due to the probing behavior of insects. The fluctuations in voltage and electrical resistance thus recorded as waveforms, which can be displayed on a computer monitor, are matched to specific feeding events. This technique provides detailed information regarding stylet activities, its tip position in plant (may it be intra or extracellular) on the basis of recorded signal potential (Tjallingii 1985). The major waveforms distinguished in EPG recordings consists of intercellular stylet activity, intracellular puncture by stylets, salivation on sieve element, feeding on phloem sieve element, xylem sap ingestion (Tjallingii and Esch 1993, Walker 2000).

Moreover, distinctive characteristics of EPG waveforms produced by insect feeding has been categorized into three behavioral phases; i) pathway phase, ii) phloem or sieve element phase and iii) xylem phase (Reese et al., 2000; Tjallingii, 2006). Aphids conduct a series of activities during the pathway phase such as puncturing the cell, inserting into and withdrawing the stylet from the cell to locate and sample the sieve element which may serve to discriminate hosts from non-hosts (Jiang and Walker, 2001). The sieve element phase comprises a salivation period and phloem ingestion followed by salivation (Tjallingii, 2006). The xylem phase includes water intake by the aphids (Spiller et al., 1990). EPG study also allows to locate plant factors affecting aphid feeding behavior based on stylet penetration rate (van Hoof 1958, Gabrys et al. 1997). For example, shorter probes less than 30 seconds indicate factors in the epidermis causing stylet withdrawal whereas longer probes ranging from 30 seconds to 3 minutes indicate stylet withdrawal from mesophyll tissue (Gabrys et al. 1997, Schwarzkopf et al. 2013).

The intracellular stylet punctures of epidermal and mesophyll cells by aphids prior reaching to phloem can be pictured as potential drops using EPG (Martin et al. 1997). Potential drop occurs due to the fluctuation in transmembrane potential in plant cells and are correlated

with brief (3-15 s) intracellular punctures (Tjallingii 1988). Each potential drop has been further characterized into 3 distinct phases; phase-I, II and III. Phase II has been considered as truly intracellular and further divided into 3 subphases, subphase II-1, II-2 and II-3 (Tjallingii 1985, Martin et al. 1997). Subphases II-1 and II-3, which have been correlated with the egestion and ingestion events by aphids, are further associated respectively with inoculation and acquisition of plant viruses, especially for non-persistent viruses (Powell et al. 1995, Martin et al. 1997, Powell 2005). The frequency of potential drops generated in EPG monitor has also been reported to determine the transmission efficiency of an aphid species (Powell et al. 1991). Powell et al. (1995) reported a relationship between presence of phase II-3 typical pulses and potyvirus acquisition, suggesting that these pulses might be reflecting an ingestion event. Collar et al. (1997) found that the probability of *Potato virus Y* (PVY) acquisition by *Myzus persicae* in pepper (*Capsicum annuum* L.) could increase with an increment in the number of pulses within phase II-3. It was suggested that the differences observed in the morphology and duration of phase II-3 of the potential drops produced by different aphid species could be correlated with differences in their transmission efficiencies (Collar et al. 1998).

2.6. Study systems

In order to understand the effects of plant viruses on the behavior and performance of vectors and non-vectors herbivores, we sought to study three different pathosystems with an aim to add insights to the limited depth of knowledge available, especially in the sector of plant-virus and non-vector herbivore interactions.

2.6.1. *Sorghum mosaic virus* (SrMV) in grain sorghum

Sorghum: Importance, usage and production in the US

Sorghum, *Sorghum bicolor* (L.) Moench, is the third largest cereal grain crop grown in the US after corn (*Zea mays* L.) and wheat (*Triticum aestivum* L.) (NASS, USDA 2017). Sorghum provides comparative advantages of drought tolerance, resistance to mycotoxins and fungi, and survivability in relatively harsher climatic conditions; making it one of the important cereal crops in the world (US Grains Council 2016). Sorghum was planted on 2.3 million hectares and 9.3 million metric tons were harvested in 2017 (NASS, USDA 2017). The major sorghum production region in the US, also known as "Sorghum Belt", runs from South Dakota to Southern Texas with top sorghum producing states being Kansas, Texas, Oklahoma, Colorado, and Arkansas respectively in 2017 (NASS, USDA 2018). In the US, nearly one third of the sorghum crop is used for renewable fuel production, 35% is used to produce animal feed, and 42% is exported (U.S. Grain Council 2016). In 2016, US exported almost 80% of total sorghum exports in the world while China accounted for 82% of the world imports (USDA-FAS 2017).

A large number of native and invasive pests attack sorghum in the US leading to a significant reduction in grain yields. Some of the common insects that attack sorghum in the US are wireworms (Elateridae), corn leaf aphid (*Rhopalosiphum maidis* Fitch), sugarcane aphid (*Melanaphis sachhari*, Zehntner), greenbug (*Schizaphis graminum* Rondani), chinch bug (*Blissus leucopterus* Say), corn earworm (*Helicoverpa zea* Boddie), fall armyworm (*Spodoptera frugiperda* J.E. Smith), cutworm (*Agrotis ipsilon* Hufnagel), false chinch bug (*Nysius raphanus* Howard), grasshopper, sorghum webworm (*Nola cereella* Bosc), and spider mites (Tetranychidae) (K-state research and extension 2018).

Sugarcane aphid, its ecology and role in plant virus transmission

Sugarcane aphid (Hemiptera: Aphididae) has a worldwide distribution and occurs commonly as a pest of sorghum and sugarcane (*Saccharum officinarum* L.) in tropical regions of the world (Singh et al. 2004). The host range of *M. sacchari* is largely confined to the species of a few genera: *Sorghum, Saccharum. Oryza, Panicum* and *Pennisetum* (Denmark 1988). However, incidence of sugarcane aphid infesting *Setaria italica* (L.) (Wilbrink 1922), *Zea mays* (L.) (Agarwala 1985), *Cynodon dactylon* (Wilbrink 1922) or *Miscanthus chinensis* (L.) (Setokuchi 1973) have also been reported. Moreover, de Souza (2018) reported that *M. sacchari* larviposited and developed to adulthood on wheat (*Triticum aestivum*). *M. sacchari* is anholocyclic throughout most of its geographical range in tropical and sub-tropical regions but some sexual oviparae forms have been reported in India (David and Sandhu 1976). Moreover, monoecious, holocyclic forms have also been reported on sugarcane (Yadava 1966) and sorghum (David and Sandhu 1976).

The earliest detailed study of life history and biology of sugarcane aphid was reported on sorghum (van Rensburg 1973a, 1973b) and sugarcane (Setokuchi 1980). Sugarcane aphid colonies on sorghum consist of both apterae and alate individuals which are predominantly lemon-yellow colored and infest the abaxial surface of the basal leaves first and then move upwards. Some alates have been reported to possess patterned black markings along the dorsal sclerites (Blackman and Eastop 1984). Asexual reproduction is predominant with adults being viviparous females. However, sexual reproduction of sugarcane aphid on sorghum has also been reported (David and Sandhu 1976) but the environmental conditions favoring sexual reproduction is not clearly understood. Sugarcane aphid has four nymphal stadia and its life cycle completes in 4.3-12.4 days (Chang et al. 1982). Adults normally live for 10-37 days

depending on environmental conditions (van Rensburg 1973a). Adult females produce up to 68 nymphs with an average of 34 nymphs in their lifetime (Meksongsee and Chawanapong 1985). Alate aphids have a short life expectancy and produce fewer nymphs as compared to apterae aphids (van Rensburg 1973a). Under screen-house conditions, sugarcane aphids are reported to produce an average of 56 generations annually (Chang et al. 1982). However, the life span and population density are affected by fluctuating temperatures and rainfall patterns (Chang et al. 1982, de Souza 2018). De Souza (2018) reported that *M. sacchari* individuals reared in excised sorghum leaves reached adulthood faster at temperatures of 25 °C to 30 °C as compared to lower or higher temperatures. Similarly, *M. sacchari* females produced more nymphs (49.8) at 20 °C than at 15 °C (36.4 nymphs) and 25 °C (40 nymphs). *M. sacchari* females had the lowest nymphal production at 30 °C and 32 °C, 4.1 nymphs and 5.1 nymphs respectively (de Souza 2018). Sugarcane aphid, though reported in Hawaii in 1896, was first documented in the continental US in Florida on sugarcane in 1977 (Mead 1978) and then in Louisiana in 1999 (White et al. 2001). Aphid populations during these infestations would peak during summer and then subside by winter. Moreover, sugarcane aphid was considered a pest of sugarcane only until 2013, when outbreaks of *M. sacchari* were observed causing economic damage on sorghum (Armstrong et al. 2015). Since the pest outbreak in 2013, many sorghum producing regions in the US have experienced higher infestations of sugarcane aphid while populations have remained moderate on sugarcane (Medina et al. 2017). This sudden change in the pest status of sugarcane aphid from a minor pest of sugarcane to a major pest of sorghum led to mainly two different hypotheses regarding the recently observed population of sugarcane aphid; i) introduction of a new biotype specialized on sorghum or ii) host preference shift in pre-existing sugarcane aphid populations (Nibouche et al. 2018). However, Nibouche et al. (2014) reported that there was a

limited genetic diversity in the sugarcane aphid populations across the US. Recently, Nibouche and colleagues (2018) published a report stating that a new invasive genotype which has a low genetic diversity and consists of a dominant clonal lineage, MLL-F, is responsible for the rapid spread and infestation in the sorghum growing regions of the US after 2013. They used microsatellite markers and COI sequencing to evaluate the genetic diversity of sugarcane aphid populations collected pre (during 2007-2009) and post 2013 (2013-2017) and found that both populations are genetically distinct (Nibouche et al. 2018).

Since its first detection in sorghum near Beaumont, Texas, in June 2013, sugarcane aphid has expanded its geographic range rapidly, reaching 24 US states by 2018 (EDDSMaps 2018). Populations of sugarcane aphid are now commonly encountered on sorghum plants from emergence to harvest. Rapid and widespread growth of sugarcane aphid on sorghum in North America has been associated with several factors such as overwintering survival on ratoon and remnant sorghum or perennial alternate hosts like Johnson grass, wind-aided long-distance dispersal of alate individuals (Bowling et al. 2016). The aphids overwinter in Southern Texas and can be swept up north with increasing temperatures (Michaud 2016, Bowling et al. 2016). In the US, observations in the field indicate that sugarcane aphid is predominantly anholocyclic, parthenogenic and viviparous (Bowling et al. 2016). The population of sugarcane aphid can be 100% females, mature in 4 to 12 days and reproduce asexually producing between 34-to-96 nymphs per individual depending upon temperature and nutrition (Singh et al. 2004). Accelerated population growth can be observed during warm and dry weather, a commonly encountered climatic conditions in majority of sorghum producing regions in North America (Bowling et al. 2016). Sugarcane aphids feed by sucking on the sap of sorghum plants. Symptoms of feeding include purpling of leaves, chlorosis, and then ultimate necrosis of plant

leaves. Effects of feeding depends upon the level and stage at which the infestation occurs. Earlier infestation before booting might actually kill young plants while later infestation during panicle initiation can prevent proper grain fill, resulting in a reduction of grain yield. Moreover, indirect effects can occur due to excretion of honey dew in excess by a large population that promotes sooty mold growth and leads to reduction in photosynthetic ability of the plants. In addition to that, losses during harvesting have also been reported due to sticky residue coating the inside of the combine and making it difficult for the machine to separate out the seed from the stalk and leaves, leading grain to ride over and be lost on the ground (Villanueva et al. 2014, Bowling et al. 2016).

The effects of sugarcane infestation on sorghum have been estimated in terms of both direct and indirect loss. In Louisiana, 27% reduction in sorghum yield was reported in 2014 (Kerns et al. 2015). Kerns et al. (2015) estimated a 22% reduction in harvest speed with more than 40 hours of pauses due to machinery breakage. An estimated total impact of $7.6 million due to sugarcane aphid was reported in 2014 in Louisiana alone, with damaging infestations present in 85% of the grain sorghum acreage in the state (Brewer and Gordy 2016). Total sorghum acreage in Louisiana decreased by 33% in 2018 (lowest planted in 56 years) as compared to 2017 while area harvested was reduced by 31% (USDA, NASS 2018).

Besides the direct injury due to feeding and honey dew secretion, sugarcane aphids have also been found to vector *Millet red leaf virus* (MRLV) (Pei and Hsu 1958, Black and Eastop 1984), a persistently transmitted plant virus and *Sugarcane yellow leaf virus* (ScYLV), a semi-persistently transmitted virus (Schenck and Leherer 2000). Schenck and Lehrer (2000) reported a 73.5% transmission of ScYLV by a single *M. sacchari* to wheat. Transmission of *Sugarcane mosaic virus* (SCMV), a non-persistently transmitted potyvirus by sugarcane aphids have been

reported in many instances (Bhargava et al. 1971, Kondaiah and Nayudu 1984, Setokuchi and Muta 1993, Singh et al. 2005, Deshmukh 2008). Yang (1986) reported that both sugarcane aphid and corn leaf aphid (*Rhopalosiphum maidis* Fitch) transmitted *Sugarcane mosaic virus* (SCMV) successfully between sorghum, corn, and sugarcane. Although sugarcane and sorghum are the preferred hosts, the host range of virus transmission by *M. sacchari* is more extensive and it includes ScYLV transmission in wheat, rice (*Oryza sativa* L.), oats (*Avena sativa* L.), and barley (*Hordeum vulgare* L.) (Schenck and Lehrer 2000). Successful transmission of ScYLV by a group of 10 to 13 *M. sacchari* individuals was also reported in maize (*Zea mays* L.) (ElSayed 2013).

***Sorghum mosaic virus*: host range, potential vectors and symptomatology**

Sorghum mosaic virus (SrMV) (Family: *Potyviridae*, Genus: *Potyvirus*) is a single stranded RNA virus consisting of a flexuous filamentous particle c. 750 nm long (Koike and Gillaspie 1976, Jilka 1990). SrMV displays distinctive symptoms on differential hosts depending upon different strains such as severe chlorosis and necrosis (strain I), mild mottle and mild mosaic (strain H), and severe red-leaf symptoms (strain M) (Tippett and Abbott, 1968), and causes yield losses in sugarcane, maize, and sorghum as high as 50% (Grisham et al., 2007, Xu et al. 2008). Transmission and spread of SrMV to sugarcane in the field has been primarily attributed to aphid vectors and infected stalk cuttings with mechanical inoculation being important in the greenhouse and laboratory research (Rott *et* al. 2008, Xu et al. 2008). SrMV is not known to be seed transmitted in sorghum (Koike and Gillaspie, 1976). Hence, vector transmission remains the only viable option for SrMV transmission in sorghum fields. SrMV was originally mistaken as a strain of *Sugarcane mosaic virus* (SCMV) (Family: *Potyviridae*, Genus: *Potyvirus*) owing to their very similar host ranges, similar symptom expression in many hosts, common aphid

vectors, and serological interrelatedness (Shukla et al., 1992). However, molecular studies have shown that SCMV and SrMV are genetically diverse and now SrMV is considered a separate virus (Shukla 1992, Yang and Mirkov 1997, Chen 2002). Though numerous studies have documented the transmission efficiency of SCMV by different species of aphids on sorghum (Singh et al. 2005), there have been no studies on the transmission efficiency of SrMV by different species of aphids feeding on sorghum because SrMV had been, until recently, thought to be a strain of SCMV. Therefore, the potential vectors of SrMV are unknown. Singh et al. (2005) reported the transmission of SCMV by different species of aphids from sorghum to sorghum at different efficiencies; *A. gossypii* (30%), *M. sacchari* (30%), *M. persicae* (50%), and *R. maidis* (70%). However, owing to the very close interrelatedness between SCMV and SrMV, a similar mode of transmission between these two viruses by different species of aphids can be hypothesized.

2.6.2. *Cucumber mosaic virus* and *Sunn-hemp mosaic virus* in cowpea

Cucumber mosaic virus (CMV) is a type member of genus *Cucumovirus*, belongs to virus family *Bromoviridae* and infects more than 1200 plant species in over 100 families of monocots and dicots worldwide (Palukaitis et al. 1992, Zitter and Murphy 2009). Described for the first time in in cucumber (*Cucumis sativus* L.) in 1916, it is now known to infect many agricultural and horticultural crops in both temperate and tropical climates worldwide (Zitter and Murphy 2009). CMV has one of the extensive host range among plant viruses, and possesses a large number of isolates (Zitter and Murphy 2009, Jacquemond 2012). Moreover, CMV is an important model system for research due to some salient features such as easy mechanical transmissibility and strong virus accumulation in infected hosts, which allows for easy purification (Palukaitis et al. 1992, Jacquemond 2012).

CMV virions are icosahedral particles 29 nm in diameter and its genome is composed of three single stranded positive sense RNAs (Jacquemond 2012). CMV was first reported in cowpea (*Vigna unguiculata* (L.) Walp.) by Robertson (1966) in Nigeria (Abdullahi et al. 2001). Symptoms of CMV infection in cowpea consist of leaf curl, leaf distortion and green mosaic, blistering, and a zipperlike roughness along the main veins (Zitter and Murphy 2009).

Sunn-hemp mosaic virus (ShMV) is a type member of genus *Tobamovirus* and family *Virgaviridae* and infects many crops in the Family Leguminosae (Boswell and Gibbs 1983). Previously known as a strain of *Tobacco mosaic virus*, Kassanis and Varma (1975) re-designated this virus as *Sunn-hemp mosaic virus*. ShMV virions are rod-shaped particles 300 nm long and its genome is composed of a single stranded positive sense RNA (Kassanis and Varma 1975). Symptoms of ShMV infection in cowpea consists of severe mosaic, puckering, blistering and malformation of leaves with enations on the underside of the leaves (Lister and Thresh 1955).

Both CMV and ShMV are non-persistently transmitted by aphids and their effects on non-vector herbivores are poorly understood. Both CMV and ShMV infect cowpea plants causing mosaic symptoms, stunted growth, and eventual yield loss (Arogundade et al. 2009, Pio-Ribeiro et al. 1978). However, the effects of these viruses on non-vector herbivores of cowpea are unknown. Two common non-vector herbivores in cowpea, soybean looper and fall armyworm, were chosen to study the effects of CMV and ShMV infection on their performance and growth.

Soybean looper, *Chrysodeixis includens* (Walker) [Lepidoptera: Noctuidae], is an important polyphagous pest which feeds on many agronomic and vegetable crops and is known to infest 31 different hosts (Herzog 1980, Moonga and Davis 2016). It is one of the most important and difficult pests to manage owing to their ability to consume massive amounts of

foliage (Mascarenhas and Boethel 1997). Similarly, fall armyworm, *Spodoptera frugiperda* (Smith) [Lepidoptera: Noctuidae], is also an important crop defoliator which displays a very wide host range feeding on more than 80 species in 23 families (Pashley 1988) including many grasses and crops such as alfalfa (*Medicago sativa* L.), soybean, corn, rice, sorghum, sugarcane, and cotton (Walton and Luginbill 1916, Hinds and Dew 1951). Historically, fall armyworm has been one of the primary pests of field corn in southern Unite States. (Pitre and Hogg 1983, Buntin 1986). A study to understand the effects of CMV and ShMV-infected cowpea on soybean looper and fall armyworm larval growth and oviposition preference by adults was conducted and results are discussed in this dissertation.

2.6.3. *Bell pepper endornavirus* in bell pepper

Plant viruses maintain a parasitic relationship with its hosts and incur negative effects to their hosts usually causing diseases except for some viruses, called persistent viruses, which do not exhibit apparent symptoms (Roossinck 2010). Plant viruses within families *Amalgaviridae, Chrysoviridae, Endornaviridae, Partitiviridae*, and *Totiviridae* are listed within persistent plant viruses (Chen et al. 2016, Fukuhara et al. 2012, Sabanadzovic and Valverde 2011, Sabanadzovic et al. 2009).

Endornaviruses belong to the genus *Endornavirus* in the family *Endornaviridae* and infect a wide variety of hosts including plants, fungi and oomycetes (Hacker et al. 2005, Okada et al. 2013, Li et al. 2014). Endornaviruses are currently categorized into two genera based on their genome size and unique domains; *Alphaendornavirus*, which infect plants, fungi and oomycetes, and *Betaendornavirus*, which infect ascomycete fungi (Adams et al. 2017). Many economically important crops such as beans, cereals, cucurbits, and peppers are infected by endornaviruses (Pfeiffer 1998, Coults 2005, Valverde and Gutierrez 2007). Generally, only

select cultivars of these crops have been shown to be infected by endornaviruses except for bell pepper (*Capsicum annuum* L.) and melon (*Cucumis melo* L.) in the US, which have been reported to be infected almost 100% (Valverde et al. 1990, Okada et al. 2011, Sabanadzovic et al. 2016). Many genotypes of common bean (*Phaseolus vulgaris* L.) have been reported to be doubly infected by two endornaviruses, *Phaseolus vulgaris endornavirus 1* (PvEV1) and *Phaseolus vulgaris endornavirus 2* (PvEV2) (Okada et al. 2013, Khamkhum et al. 2015). Plants infected by endornaviruses are normal phenotypically and do not display any characteristic viral symptoms (Okada et al. 2011, Song et al. 2013). In addition to plants, several species of fungi are also reported to be infected by endornaviruses such as *Alternaria brassicola, Helicobasidium mompa,* and *Tuber aestivum* (Osaki et al. 2006, Stielow et al 2011, Shang et al. 2015).

Endornaviruses have a single, linear double stranded RNA genome (9.8-17.6 kbp), are generally transmitted at a high rate only through seeds, and are present in a very low copy number (Moriyama et al. 1996, Horiuchi and Fukuhara 2004, Valverde and Gutierrez 2007). Endornaviruses lack both coat protein and movement proteins and are solely comprised of naked RNA (Roossinck et al. 2011). Endornaviruses contain only one open reading frame which normally encodes a single polypeptide, presumed to be processed by virus-encoded proteinases (Okada et al. 2011). Genomes of all completely sequenced endornaviruses consist of conserved motifs of RNA-dependent RNA polymerase and viral RNA helicases (Gibbs et al. 2000).

BPEV (*Bell pepper endornavirus*), like other endornaviruses, shows no typical viral disease symptoms on bell peppers (Aguilar-Melendez et al. 2009). BPEV infected plants lack apparent symptoms and virus cannot move cell-to-cell but are found at constant concentrations in every tissue and at all developmental stages of plants (Okada et al. 2011). Endornaviruses possibly interact with plant hosts in many ways: i) parasitic, using host resources for their

replication, ii) mutualistic, a possibility looking at the high rate of vertical transmission from parent to progeny suggesting a selection for the endornavirus infection, or iii) commensalistic, benefitting the virus while no effects on their hosts (Herschlag 2017).

BPEV in bell peppers were reported to be present in all tested bell pepper cultivars by Valverde and Fontenot (1991). It appears that plant breeders, oblivious of the presence of endornaviruses in the germplasm, favored endornavirus-infected genotypes, suggesting an inadvertent selection and introduction of virus infected genotypes in bell pepper growing regions. Since BPEV is transmitted vertically and is present at constant concentrations in almost all commercial bell pepper cultivars, it may be providing a benefit to the host. One of the possible benefits that endornaviruses render to the host might include tolerance or resistance to biotic and abiotic agents. Therefore, endornaviruses may have evolved a symbiotic relationship with their hosts to tolerate stresses. Thus, a study looking at the possible benefit of BPEV to bell pepper in coping with a biotic agent, green peach aphid, was conducted and discussed in this dissertation.

2.7. References

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CHAPTER 3: THE FEEDING BEHAVIOR AND VECTOR POTENTIAL OF *MELANAPHIS SACCHARI* IN TRANSMITTING *SORGHUM MOSAIC VIRUS* TO SORGHUM

3.1. Introduction

Aphids are known to differ in their ability to transfer plant viruses depending upon the species, environmental conditions, and the nature of virus (Ng and Perry, 2004). This ability, also known as transmission efficiency, is defined as the frequency with which a single aphid can transmit a plant virus under standard conditions (Gibbs and Gower 1960, Gildow et al. 2008). Studies have suggested that transmission efficiency varies among aphid species, biotypes, life stages, different strains of a viruses, and their host plants (Gill 1970, Gibson et al. 1988, Boiteau et al. 1998, Davis et al. 2005, Cervantes and Alvarez 2011, Mello et al. 2011, Mondal et al. 2015). Gill (1970) reported that *Rhopalosiphum maidis* (Fitch) nymphs were more efficient in transmitting an isolate of *Barley yellow dwarf virus* than adults in oats (*Avena sativa*). Mondal et al. (2015) reported that green peach aphid (*Myzus persicae* Sulzer) transmitted three strains of *Potato virus Y* (PVY^{NTN}, PVY^{o} and $PVY^{N:O}$) at different efficiencies among strains and at a greater efficiency than bird cherry oat aphid (*Rhopalosiphum padi* L.). The differences in transmission efficiency of *Bean yellow mosaic virus* among two pea aphid (*Acyrthosiphon pisum Harris*) biotypes was reported earlier by Sohi and Swenson (1964). Moreover, non-colonizing aphids are reported to be more important vectors of non-persistently transmitted viruses (Racah et al. 1985, Summers et al. 1990, Peters et al. 1990).

Sugarcane aphid, *Melanaphis sacchari* (Zehntner), is an important invasive pest in the US sorghum (*Sorghum bicolor L.*). Historically, sugarcane aphid has been listed under at least two names, *Aphis sacchari* (Zehntner) (Zimmerman 1948), and *Longiunguis sacchari* (Zehntner) (Eastop 1965). Moreover, various genera were synonymously used for *Melanaphis* Van der Goot

(1917) such as *Yezabura* Matsumura (1917), *Geoktapia* Mordovilko (1921), *Nevsikia* Mordovilko (1932), *Piraphis* Borner (1932), *Masraphis* Soliman (1938), *Schizaphideilla* Hille Ris Lambers (1939), and *Longiunguis* Van der Goot (1977) (Eastop and Hille Ris Lambers 1976, Singh et al. 2004). Roy Chaudhari and Banerjee (1974) named sugarcane aphid as *M. sacchari* (Zehntner), a name commonly used in literature since then. Sugarcane aphid has been reported to vector and transmit different plant viruses at different efficiencies. *Millet red leaf virus* (MLRV), a persistently transmitted plant virus was found to be transmitted by *M. sacchari* to fox millet (*Setaria italica* L.) at 20% transmission efficiency (Pei and Hsu 1958). Similarly, Schenck (2000) reported that *M. sacchari* could transmit *Sugarcane yellow leaf virus* (SCYLV), a semi-persistently transmitted virus, to wheat (*Triticum aestivum* L.) with a transmission efficiency of 73.5%. *Sugarcane mosaic virus* (SCMV), a non-persistently transmitted virus, was found to be vectored and transmitted at 30% transmission efficiency by *M. sacchari* to sorghum (Singh et al. 2005). Reports by Kondaiah and Nayudu (1984) stated a transmission efficiency of 65.0% for SCMV by *M. sacchari* to sorghum variety "Rio". Also, transmission efficiency of SCMV by different species of aphids has been documented on sorghum (Singh et al. 2005) but there have been no studies on the transmission of SrMV by aphids feeding on sorghum, because SrMV had been, until recently, thought to be a strain of SCMV. Singh et al. (2005) reported the transmission of SCMV by different species of aphids from sorghum to sorghum at different efficiencies; *Aphis gossypii* (Glover.) (30%), *M. sacchari* (Zehntner) (30%), *Myzus persicae* (50%) and *Rhopalosiphum maidis* (Fitch) (70%).

Mosaic symptoms in sugarcane are attributed to a complex of five related but distinct species of potyviruses: *Sugarcane mosaic virus* (SCMV), *Sorghum mosaic virus* (SrMV), *Maize dwarf mosaic virus* (MDMV), *Johnsongrass mosaic virus* (JGMV) and *Zea mosaic virus*

(ZeMV) (Handley et al. 2001, Rott et al. 2008). Among these viruses, only SCMV and SrMV are reported to infect sugarcane and are thus regarded as the causal agents of sugarcane mosaic (Grisham 2000, Rott et al. 2008). Sugarcane mosaic, historically, has been a serious disease problem and caused a near collapse of the sugarcane industry in Louisiana after being superimposed to already established diseases such as Pythium root rot and red rot in the 1920s (Abbott 1960).

Most of the aphid-borne potyviruses infecting different members of *Poaceae* including sugarcane, until recently, were thought to be either sugarcane mosaic or maize dwarf virus (Grisham 1994). However, speciation of potyviruses based on amino-acid sequence homology and serological relationships of the coat proteins have allowed categorization of previous included strains of *Sugarcane mosaic virus* or *Maize dwarf virus* into four distinct viruses: SCMV, JGMV (*Johnson grass mosaic virus*), SrMV (*Sorghum mosaic virus*) and MDMV (*Maize dwarf mosaic virus*) (Shukla et al. 1989, Shukla and Teakle 1989, Ford et al. 1989, McKern et al. 1990).

Sorghum mosaic virus (SrMV) (Family: Potyviridae, Genus: Potyvirus) is an important potyvirus that causes mosaic disease in sugarcane, maize, sorghum, and other *Poaceae* species (Grisham et al. 2007, Xu et al. 2008). In susceptible sorghum cultivars, mosaic causes reduction in grain and forage production, and displays distinctive symptoms depending upon different strains such as severe chlorosis and necrosis (strain I), mild mottle and mild mosaic (strain H), or severe red-leaf symptoms (strain M) (Tippett and Abbott 1968, Silva et al. 2012). SrMV, though it infects various grain crops and grasses, is more prevalent in sugarcane than sorghum in field conditions in Louisiana (Dr. Jeff Hoy, personal communication). However, in the United States, *Sugarcane yellow leaf virus* (ScYLV) (Luteoviridae: *Polerovirus*), a disease historically related

with sugarcane, was detected in 41% of sorghum plants tested in Florida (Wei et al. 2016). Moreover, de Souza (2017) reported the incidence of JGMV infecting sorghum in Brazil. Therefore, there is a possibility of a host switch or host expansion by viruses historically infecting sugarcane into other related crops and grasses.

Understanding the feeding behavior of aphids is crucial in elucidating virus transmission. Before feeding is initiated, aphids select a host in a complex process involving different stimuli and responses (Fereres and Moreno 2009). Antennae of aphids bear many sensilla which are used in chemoreception of leaf surfaces (Park and Hardie 2004). In addition, the tips of the proboscis in aphids have tactile receptors that scan surfaces of potential hosts and allow them to distinguish the outline of veins, preferred feeding sites of aphids (Tjallingii 1978b). Aphids then probe into the plant with the help of their stylet mouth parts. Mouth parts of aphids consist of maxillae and mandibles which are elongated into a stylet bundle that facilitates the penetration of plant tissue and reach the phloem whereas the tip of the labium helps to guide stylet penetration (Forbes 1977).

Aphids produce two different types of saliva during feeding that mediate their interactions with plants which are watery and jelling saliva (Miles 1989). During the process of plant tissue penetration, aphids usually discharge a salivary sheath that jells around the stylets forming a stylet sheath to facilitate penetration, seal puncture sites on cell membranes, isolate plant tissues from mouth parts, and prevent plant reaction at feeding sites (Pollard 1973, Felton and Eichenseer 1999). The salivary sheath commonly ends in the phloem specifying that aphids feed on the sieve element contents (Pollard 1973). Watery saliva is secreted during cell penetration and ingestion by aphids. If the aphids come up against resistant cultivars or non-hosts, they tend to cease feeding shortly after phloem penetration, withdraw their stylets, and

abandon the plant; indicating that phloem sap of non-hosts or resistant plants is nutritionally unsuitable (Dixon 1998).

Aphids usually feed within the plant tissues, an opaque food substrate, and cannot be observed directly. Therefore, special techniques are required to study their feeding behaviors (Walker 2000). The most important advancement in the development of specific techniques to study feeding behavior was the development of electronic feeding monitor by McLean and Kinsey (1964). This technique was modified and improved by Tjallingii (1985) and is currently named as electrical penetration graph (EPG) technique. Most of the advances since 1960s in hemipteran feeding behavior have been accomplished through the use of EPG technique (Walker 2000). Two types of EPG systems have been devised, those that McLean and Kinsey originally developed use AC (alternating current) circuitry and are referred to as AC EPG systems (Kinsey and McLean 1987, Backus and Benett 1992), and those that came as a modification to the original monitor by substituting AC circuitry with DC (direct current) circuitry and referred to as DC EPG systems (Tjallingii 1985). The changes occurring in electrical resistance in the probing insect and the plant is measured by both EPG systems. Moreover, DC EPG system also measures voltage changes originating in the plant and probing insect along with changes in resistance. (Tjallingii 1978a).

The EPG monitor consists of two electronic components, a voltage source and an input resistor which are connected electrically by the help of an output and an input receptacle (Backus and Benett 1992). The output receptacle and input receptacle is directly connected to the voltage source and the input resistor respectively (Walker 2000). The insect and the plant complete the circuit in EPG system by connecting a wire from the output to the plant and input to the insect (Walker 2000). The input receptacle makes contact with the insect by connecting to a thin gold

or platinum wire (2.5 to 25 μm in dia.) which is glued to the insect's dorsum with a small drop of electrically conducive adhesive such as silver paint. The wire glued to the insect's back is thin and flexible enough to allow insects to move freely during feeding. The output wire is connected with the plant through copper wire inserted in the potting mixture.

As EPG consists of a host plant and a piercing-sucking insect attached in an electrical circuit through a wire, the circuit is completed as soon as the mouthparts of insect penetrates the plant. Penetration activities generate electrical events inside the stylet canals or at its extremities (Tjallingii 1988). The alterations in voltage and electrical resistance are recorded as waveforms, and displayed on a computer monitor. These waveforms are matched to specific feeding events. Aphid behaviors recorded by EPGs can be categorized as probing (stylets inserted inside the plant tissue) and non-probing. Different activities within probing such as intercellular stylet activity, intracellular puncture, salivation in sieve elements, feeding on phloem sieve elements, and xylem ingestion can be recognized as distinct waveform patterns (Tjallingii and Esch 1993, Walker 2000). Waveforms generated by a typical DC monitor can be listed as nonpenetration or nonprobing (NP), stylet pathway phase (A, B, C, potential drop (pd), and F), sieve element phase (X, E1, and E2) and xylem phase (G) (van Helden and Tjallingii 2000). Moreover, distinctive characteristics of EPG waveforms produced by aphid feeding can be categorized into three behavioral phases: pathway phase, phloem or sieve element phase and xylem phase (Reese et al. 2000, Tjallingii 2006). During pathway phase, aphids conduct activities such as puncturing the cell, inserting into and withdrawing the stylet from the cell to locate and sample the sieve element (Jian and Walker 2001). The phloem phase consists of a salivation period and phloem ingestion (Tjallingii 2006). Water intake by aphids occurs during xylem phase (Spiller et al. 1990).

Intracellular stylet punctures of epidermal and mesophyll cells by aphids prior to reaching phloem can be envisioned as potential drops (pds) using EPG (Powell et al. 1995). Fluctuation in transmembrane potential in plant cells cause potential drops and these are correlated with brief (3-15 s) intracellular punctures (Tjallingii 1988). Each potential drop is further delineated into 3 distinct phases; phase-I, II and III. The first phase (I) occurs during the initial puncture of the membrane and includes the drop of potential to intracellular signal level; second phase (II) consists of the maintenance of this potential while the third phase (III) represents the return of the potential to its original extracellular level which is supposedly caused by the withdrawal of the stylet from the plasma membrane (Powell et al. 1995). Among these three phases, phase II is considered as truly intracellular and further sub-divided into 3 sub-phases, sub-phase II-1, II-2, and II-3 (Tjallingii 1985, Martin et al. 1997). Sub-phases II-1 and II-3 are correlated with egestion and ingestion events and further linked respectively, with inoculation and acquisition of plant viruses, especially for non-persistently transmitted viruses (Powell et al. 1995, Martin et al. 1997, Powell 2005).

Transmission of non-persistently transmitted viruses has been correlated with various activities during potential drop and subsequent phases within the potential drop. Puncture of cell membrane during the penetration of epidermis (potential drops) were linked with transmission of two potyviruses, *Beet mosaic virus* (BMV) and *Potato virus Y* (PVY) (Powell 1991). Powell et al. (1992) reported a positive correlation between frequency of potential drops and transmission efficiency of PVY by two aphid species, *Brachycaudus helichrysi* Kltb (15%) and *Drepanosiphum platanoidis* Schrank (1%). Moreover, a relationship between presence of phase II-3 typical pulses (archlets) and acquisition of potyvirus was reported, suggesting that these pulses might be indicating an ingestion event (Powell et al. 1995). Collar et al. (1997) reported

that the probability of PVY acquisition in pepper (*Capsicum annuum* L.) by *Myzus persicae* could increase with an increase in the number of archlets within phase II-3. The differences observed in the morphology and duration of phase II-3 of the potential drops were also suggested to correlate with transmission efficiencies of different aphid species (Collar et al. 1998).

Since 2013, outbreaks of *M. sacchari* have caused economic loss to sorghum in the US and its potential to vector plant viruses adds a new dimension to the injury it can cause in crop production systems. Once established, a colony in the sorghum field can grow exponentially and as many as 10,000 individuals have been reported on a single plant in south Texas (Bowling et al. 2016). The sheer numbers of individuals of *M. sacchari* pose a huge risk for virus disease epidemics even if the efficiency of virus transmission is very low in field conditions. Since various studies suggest *M. sacchari* to be a vector of plant viruses (Pei and Hsu 1958, Kondaiah and Nayudu 1984, Schenck 2000, Singh et al. 2005), we decided to study the transmission ability of SrMV by *M. sacchari* to sorghum. Owing to very close interrelatedness between SCMV and SrMV, we hypothesized a similar mode of transmission between these two viruses by different species of aphids. Thus, we designed laboratory experiments to understand the potential of *M. sacchari* to vector SrMV in sorghum. Virus transmission efficiency of sugarcane aphid was studied in comparison to a model vector for potyviruses, the green peach aphid. Finally, we conducted EPG studies to characterize the stylet penetration behaviors of *M. sacchari* and *M. persicae* on virus-infected and non-infected sorghum cv. Rio to determine if there are differences in behaviors that could correlate with their respective transmission efficiencies of SrMV.

3.2. Materials and Methods

3.2.1. Aphid colony

Sugarcane aphids used in these experiments were from a colony, designated as LSU-SCA14, founded from a single apterae field collected from sorghum at the Louisiana State Agricultural Center, Dean Lee Research Station, Alexandria, LA in July 2014 by J. A. Davis. The colony was maintained on Pioneer 85G85, a sorghum hybrid resistant to greenbug (Pioneer Hi-Bred International, Inc., Johnston, IA), planted in plastic pots 10 cm in diameter containing sterile potting mix (Miracle-Gro Organic Choice Garden Soil, Marysville, OH) and 5g Osmocote (14:14:14), a slow releasing fertilizer (The Scotts Company, Marysville, OH). The plants were grown in growth chambers (Percival Scientific, Perry, IA) maintained at 25 ± 2 °C, 50 ± 5% RH and a photoperiod of 14:10 (L: D). The sugarcane aphid colony was maintained under laboratory conditions in screened cages at room temperature (20-22 °C) and a 14:10 (L: D) photoperiod.

A single apterous *M. persicae* collected from eggplant (*Solanum melongena* L.) in 2009 was used to establish the green peach aphid colony. This colony was maintained in screened cages (30 x 30 x 30 cm, constructed using Plexiglass plastic sheet and fabric made of nylon mesh) and reared on mustard (*Brassica juncea* L.) cv. Tendergreen (W. Atlee Burpee and Co., Warminster, PA), under laboratory conditions at room temperature (20-22 °C) and a 14:10 (L: D) photoperiod. Mustard plants were planted in plastic pots 10 cm in diameter (Dillen Products, Middlefield, OH) containing sterile potting mix (Miracle-Gro Organic Choice Garden Soil, Marysville, OH) and 5g Osmocote (14:14:14) (The Scotts Company, Marysville, OH) and grown in growth chambers (Percival Scientific, Perry, IA) maintained at 25 ± 2 °C, 50 ± 5% RH and a photoperiod of 14:10 (L: D). A cohort of 5 to 10 aphids was placed on fresh plants to set up a new colony every 2 to 3 weeks.

3.2.2. Virus source and maintenance

SrMV infected sorghum cv. Rio, tested via RT-PCR (Reverse Transcription- Polymerase Chain Reaction), was obtained from the Sugarcane Pathology Laboratory (Department of Plant Pathology and Crop Physiology, LSU, under the direction of Dr. J. Hoy) and the virus source was maintained in the greenhouse through mechanical inoculation. Mechanical inoculation was carried out by grinding leaf tissues from infected plants showing mosaic symptoms in extraction buffer (0.01 M phosphate buffer with pH 7.0 and 1.0% sodium sulphite) at the ratio of 1:2 (weight by volume) by using a mortar and pestle. A foliar abrasive, 400 mesh carborundum powder (Fisher Scientific, Hampton, NH) was dusted on healthy plants at the 3-4 leaf stage (approx. 2 weeks after sowing) before inoculation. Then, an absorbent cotton ball dipped in virus extracted buffer solution was used to gently rub leaves of healthy test plants. After inoculation, plants were allowed to grow and symptoms were observed and tissue were tested with DAS-ELISA (Double Antibody Sandwich- Enzyme Linked Immunosorbent Assay) as per manufacturer's protocol (Kit No. DEIAPV254, Creative Diagnostics, Shirley, NY, USA). Non-symptomatic plants as well as plants testing negative through ELISA were discarded. Non-infected host plants (control) used for the EPG experiments were inoculated with virus extraction buffer only (sham-inoculated).

3.2.3. Test plants

Sorghum cv. Rio, a susceptible and differential host for SrMV (Tippett and Abbott 1968), was used for the experiments. Sorghum seeds were sown in plastic pots 10 cm in diameter (Dillen Products, Middlefield, OH) containing sterile potting mix (Miracle-Gro Organic Choice Garden Soil, Marysville, OH) and 5g Osmocote (14:14:14) (The Scotts Company, Marysville, OH) and maintained in the green house at 22 to 28 °C and varying RH 21 to 98%. Plants were grown inside tent-like cages 61 x 61 x 61 cm in dimension (Bugdorms, BioQuip Products, Inc., USA) in

order to ensure no cross transmission. Sorghum plants at 3-leaf stage (approximately 2 weeks after sowing) were used as the test plants.

3.2.4. Transmission efficiency assay

Two aphid species, *M. sacchari* and *M. persicae* were tested for their ability to transmit SrMV. Adult apterae (wingless) aphids were removed from their respective colonies using a camel's hair paint brush and placed in petri dishes (10 x 1.5 cm diameter) (Fisherbrand Petri Dishes, Fisher Scientific Co. LLC, Hampton, NH) lined with moist filter paper. Aphids were allowed a pre-acquisition fast of two hours prior to transmission assays. Aphids were gently moved to the adaxial surface of the youngest fully expanded and symptomatic leaf of the source plant and given an acquisition access period (AAP) of 5 minutes. During the AAP, aphids were observed probing with the help of a handheld magnifying glass and only those aphids that were recognized as probing on the leaf surface by arrestment activity and characteristic body posture as described by Gray and Banerjee (1999) were used for the inoculation assays. Aphids were then transferred to the youngest fully expanded leaf of an individual test plant. Aphids were given an inoculation access period (IAP) on each test plant unbound for 24 h. After IAP, feeding was terminated me°hanically by removing each aphid from the plant and manually crushing it. Test plants were transferred to the green house, sprayed with safer insecticidal soap and maintained inside cages (Bugdorms, BioQuip Products, Inc., USA) in order to avoid cross-contamination by escapees or naturally occurring potential vectors. Test plants were observed for symptom expression for 4 weeks following inoculation and leaf tissues were collected for virus testing through DAS-ELISA. Each species of aphid was tested singly or in groups of 3 or 5. Twenty-five plants were used per aphid species per treatment and each experiment was repeated 4 times. As a control check for virus transmission, starved non-viruliferous aphids were tested as above except the

source plant was a sham-inoculated one.

3.2.5. Virus detection

Virus detection was performed by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using polyclonal kits according to the manufacturer's protocols (Creative Diagnostics, Shirley, NY, USA). Absorbance was measured at 405 nm with a Thermo Multiskan MCC Type 355 Microplate Reader (Fisher Scientific, Hampton, NH, USA). Plants were deemed positive if optical density readings were greater than mean of negative controls (non-infected plants) added to three times their standard deviation (Sutula et al. 1986, Davis et al. 2008).

3.2.6. Electrical Penetration Graph experiments

To study probing behavior of *M. sacchari* and *M. persicae* on virus infected and sham inoculated plants, EPG experiments were conducted in a Faraday cage using a Giga 4-8 DC EPG amplifier with one Giga Ohm input resistance and an AD conversion rate of 100 Hz (Wageningen Agricultural University, Wageningen, The Netherlands) running only the first four channels. A DAS-710 Digital Acquisition Card (Keithley Instruments, Inc., Cleveland, OH) converted analog signals to digital which were visualized and recorded using WinDaq/Lite software (DATAQ Instruments, Inc., Akron, OH). Adult apterous aphids were removed from their respective colonies with the help of a fine camel's hair brush and placed in petri dishes (10 x 1.5 cm diameter) (Fisherbrand Petri Dishes, Thermo Fisher Scientific Inc., Pittsburgh, PA) lined with moist filter paper. Aphids were starved for 2 hours, just as in vector efficiency tests, prior to subjecting them to EPG. A 2-cm length of 18 µm gold wire (GoodFellow Metal Ltd. Cambridge, UK) was attached to the dorsum of the aphid with the help of silver conductive paint (Pelco Colloidal Silver no. 16034, Ted Pella, Inc., Redding, CA). Two test plants (one infected and symptomatic, and another sham inoculated) were placed randomly within the Faraday cage.

Next, two aphids (each a different species) were placed on the adaxial surface of two separate individual leaves on each plant; comprising of total 4 aphids per experiment. The feeding behavior was recorded for 30 minutes per aphid. The experiment was repeated 30 times; 30 aphids each per host type. Each set of aphids were given a fresh set of plants during each replication. Variables associated with intracellular stylet penetration behaviors, reported to be responsible for virus transmission, were recorded and evaluated. The variables evaluated were: time to first probe, duration of first probe, mean probe duration, total probe duration, number of probes per aphid, time to first potential drop (pd), pd duration, duration of sub phases (II-1, II-2, and II-3), number of archlets in subphase II-3 per pd, number of pds with archlets per probe, number of pds per probe, and total pd duration per aphid.

3.2.7. Data analysis

The transmission efficiency (TE) of each aphid species was estimated as number of infected test plants divided by total number of plants tested and expressed in terms of percentage as described by Davis et al. (2005). For groups of 3 and 5 aphids, the maximum likelihood estimator (MLE) was used (Venette et al. 2002). MLE was estimated as $1 - (1 - y/x)^{1/m}$, where y was the number of test plants positive for SrMV, x was the total number of test plants, and m was the number of aphids used per test plant. Confidence intervals (CI) for MLE were calculated using Microsoft Excel add-in *PooledInfRate* Version 3.0 (Biggerstaff 2006). Feeding behavior data were not normally distributed and were analyzed using a non-parametric statistical test (Wilcoxon/Kruskal-Wallis test) using JMP software from SAS (JMP Pro 14, SAS Inc, Cary, NC).

3.3. Results

3.3.1. Transmission efficiency assays

Sugarcane aphid failed to transmit SrMV singly or in groups (Table 3.1). None of the test plants inoculated with sugarcane aphid showed characteristic mosaic symptoms and the diagnostic test through DAS-ELISA were negative for all the samples tested. Green peach aphid transmitted SrMV at 8.2% efficiency (single aphid), 4.5% (groups of 3) and 4.2% (groups of 5). Green peach aphid was a more efficient vector of SrMV than sugarcane aphid. There was no transmission of SrMV from non-infected acquisition sources.

Table 3.1. Sugarcane aphid and green peach aphid *Sorghum mosaic virus* (SrMV) transmission tests.

Aphid species	Single/group[a]	N[b]	Source	%SrMV +	CI[c]
M. sacchari	Single	25	Non-infected	0.0	…
	Single	100	Infected	0.0	…
	Group of 3	25	Non-infected	0.0	…
	Group of 3	97	Infected	0.0	…
	Group of 5	25	Non-infected	0.0	…
	Group of 5	100	Infected	0.0	…
M. persicae	Single	25	Non-infected	0.0	…
	Single	98	Infected	8.2	3.9 to 14.8
	Group of 3	25	Non-infected	0.0	…
	Group of 3	100	Infected	4.5	2.5 to 7.4
	Group of 5	25	Non-infected	0.0	…
	Group of 5	99	Infected	4.2	2.6 to 6.3

[a] Tested in groups of 3, 5 or as individuals.
[b] Number of groups.
[c] 95% Confidence intervals of SrMV infection rates.

3.3.2. Stylet penetration behaviors on SrMV-infected and sham-inoculated host plants by two aphid species

Nonparametric Wilcoxon/Kruskal-Wallis tests revealed that the following stylet penetration behaviors by *M. persicae* and *M. sacchari* on SrMV-infected and sham-inoculated sorghum cv. Rio were statistically significant; probe duration ($\chi^2 = 10.6387$, df = 3, $P = 0.0138$), number of probes per aphid ($\chi^2 = 17.8409$, df = 3, $P = 0.0005$), time to first pd ($\chi^2 = 10.9605$, df = 3, $P = 0.0101$), pd duration ($\chi^2 = 30.0188$, df = 3, $P < 0.0001$), pd subphase II-1 duration ($\chi^2 = 86.3774$, df = 3, $P < 0.0001$), pd subphase II-2 duration ($\chi^2 = 19.0466$, df = 3, $P = 0.0003$), pd subphase II-3 duration ($\chi^2 = 27.7253$, df = 3, $P < 0.0001$), number of archlets in subphase II-3 per pd ($\chi^2 = 63.0108$, df = 3, $P < 0.0001$), number of pds with archlets per probe ($\chi^2 = 52.0851$, df = 3, $P < 0.0001$), number of pds per probe ($\chi^2 = 14.7002$, df = 3, $P = 0.0021$), and total pd duration per aphid ($\chi^2 = 7.9929$, df = 3, $P = 0.0462$) (Table 3.2). Other variables evaluated were not statistically different among treatments.

Each pair comparisons among treatments for the variables that were statistically significant was conducted using Wilcoxon post hoc test. The following stylet penetration behaviors were significantly different among aphid species on SrMV-infected sorghum; number of probes per aphid ($P = 0.0271$), time to first pd ($P = 0.0144$), pd duration ($P < 0.0001$), pd subphase II-1 duration ($P < 0.0001$), pd subphase II-3 duration ($P = 0.0003$), number of archlets in subphase II-3 per pd ($P < 0.0001$), number of pds with archlets per probe ($P < 0.0001$), and total pd duration per aphid ($P = 0.0284$). Other variables evaluated were not statistically different among aphid species on SrMV-infected host (Table 3.2).

On non-infected (Sham-inoculated) sorghum cv. Rio, following stylet penetration behaviors were significantly different among two aphid species: average probe duration ($P = 0.0037$), number of probes per aphid ($P = 0.0005$), time to first pd ($P = 0.0102$), pd subphase II-1

duration (P <0.0001), number of archlets in subphase II-3 per pd (P < 0.0001), and number of pds with archlets per probe (P = 0.0014). Other variables evaluated were not statistically different among aphid species on non-infected host (Table 3.2). While comparing the stylet penetration behavior of *M. persicae* among virus infected and non-infected hosts, there were statistically significant differences in three parameters: mean duration of pd subphase II-2 was higher on non-infected hosts as compared to infected hosts (P = 0.0006), mean number of pds with archlets per probe was higher on infected hosts (P < 0.0001), and mean number of pds per probe was also higher on infected hosts (P < 0.0001) (Table 3.2).

Table 3.2. Stylet penetration behaviors of *Myzus persicae* and *Melanaphis sachhari* on SrMV infected and sham-inoculated sorghum cv. Rio[a, b].

[a] Means followed by same letter within columns are not statistically significant (P > 0.05, Wilcoxon test).
[b] Duration measured in seconds and counts in numbers.

	M. persicae		*M. sacchari*		
EPG parameters	**Sham-inoculated**	**SrMV-infected**	**Sham-inoculated**	**SrMV-infected**	**Wilcoxon/Kruskal-Wallis (Prob > Chisq)**
Time to first probe	96.495 ± 46.026 a	132.291 ± 41.167 a	186.724 ± 46.026 a	182.986 ± 41.167 a	0.3682
Duration of first Probe	82.100 ± 46.739 a	134.553 ± 43.585 a	126.122 ± 46.739 a	103.639 ± 41.805 a	0.3422
Probe duration	114.219 ± 24.492 a	172.373 ± 24.933 a	296.619 ± 34.045 b	169.814 ± 28.878 a	0.0138
Total probe duration	651.046 ± 88.940 a	758.439 ± 79.551 a	795.478 ± 84.801 a	535.569 ± 78.006 a	0.1318
No. of probes/aphid	5.700 ± 0.514 a	4.542 ± 0.469 b	2.727 ± 0.490 c	2.957 ± 0.479 c	0.0005
Time to first pd	342.364 ± 95.999 a	334.299 ± 84.926 a	712.795 ± 95.999 b	582.339 ± 91.073 b	0.0101
Pd duration	5.130 ± 0.147 a	4.973 ± 0.144 a	4.955 ± 0.150 a	4.170 ± 0.162 b	< 0.0001
Pd II-1 duration	1.420 ± 0.036 a	1.485 ± 0.035 a	1.135 ± 0.036 b	1.069 ± 0.040 b	< 0.0001
Pd II-2 duration	1.438 ± 0.049 a	1.244 ± 0.047 b	1.510 ± 0.048 a	1.199 ± 0.053 b	0.0003
Pd II-3 duration	2.235 ± 0.124 a	2.029 ± 0.119 a	2.089 ± 0.121 a	1.515 ± 0.134 c	< 0.0001
No. of archlets in II-3/pd	1.174 ± 0.125 a	1.099 ± 0.117 a	0.055 ± 0.020 b	0.151 ± 0.046 b	< 0.0001
No. of pds with archlets/probe	0.400 ± 0.069 a	1.000 ± 0.107 b	0.066 ± 0.093 c	0.182 ± 0.089 ac	< 0.0001
No. of pds/probe	1.113 ± 0.247 a	2.032 ± 0.334 b	2.179 ± 0.355 b	1.766 ± 0.343 b	0.0021
Total pd duration/aphid	31.268 ± 4.244 a	31.142 ± 4.147 a	28.186 ± 4.147 a	18.249 ± 4.055 b	0.0462

Similarly, EPG parameters with statistically significant differences while comparing *M. sacchari* stylet penetration behavior among infected and non-infected hosts were probe duration

(P = 0.0134), total probe duration (P = 0.0426), pd duration (P = 0.0070), duration of pd subphase II-2 (P = 0.0107), duration of pd subphase II-3 (P = 0.0012), and total pd duration per aphid (P = 0.0181) (Table 3.2).

3.4. Discussion

SrMV is not reported to be seed transmitted in sorghum and therefore, vector transmission remains the only viable option in sorghum fields (Koike and Gillaspie 1976, Rott et al. 2008, Xu et al. 2008). SrMV is naturally more prevalent in sugarcane in field conditions (Rice 2018) and no severe problem due to SrMV has been reported in sorghum. However, the compendium of sorghum diseases published by the American Phytopathological Society (2nd edition, 2000) states "SrMV is reported from the Gulf Coast States, where it is a primary problem in sorghum grown near sugarcane". Sugarcane aphid is a colonizing aphid in sorghum and has recently been expanding its geographic range in the United States since its outbreak in grain sorghum in 2013 (Bowling et al. 2016). However, based on suction trap catches, Lagos-Kutz et al. (2018) have reported that sugarcane aphids appear to arrive late in the growing season in the northern states and hence are currently only a minor pest at those locations. Though earlier reports had demonstrated sugarcane aphid's ability to transmit potyviruses, especially *Sugarcane mosaic virus* (Bhargava et al. 1971, Kondaiah and Nayudu 1984, Setokuchi and Muta 1993, Singh et al. 2005, Deshmukh 2008), we failed to detect SrMV transmission by sugarcane aphid both singly or in groups under laboratory conditions. However, transmission efficiencies have been reported to vary among aphid species, biotypes within a species, life stages, different strains of a virus, and their host plants (Gill 1970, Gibson et al. 1988, Boiteau et al. 1998, Davis et al. 2005, Cervantes and Alvarez 2011, Mello et al. 2011, Mondal et al. 2015). Based on our results, we

state that sugarcane aphid is a non-vector of SrMV in sorghum. Thus, there appears to be no indication that sorghum growers need to be concerned about indirect damage that sugarcane aphid can cause in sorghum through transmission of SrMV.

Green peach aphid, however, was able to successfully transmit SrMV in sorghum in our study, hence, green peach aphid is a vector of SrMV in sorghum. The transmission efficiency of SrMV by green peach aphid (4.2 to 8.2%) in our experiments was rather low for a model vector but it is still comparable to other reports for potyviruses. For example, *M. persicae* was reported to transmit PVY with a varying efficiency of 4.7 to 71.1% (Cervantes and Alvarez 2011, Davis et al. 2005, Mondal et al. 2015); *Soybean mosaic virus* at 18.6%, *Alfalfa mosaic virus* at 5 to 19% (Symmes and Perring 2007); and *Sweet potato feathery mottle virus* (SPFMV) at 0 to 18 % (Wosula et al. 2012). Green peach aphid is a non-colonizing aphid in grain sorghum and is rarely found in sorghum fields but is abundant in sugarcane (Singh et al. 2005, Blackman and Eastop 2006). Therefore, green peach aphid could spread SrMV from sugarcane to sorghum and within sorghum fields. Even though the results under laboratory conditions indicate that green peach aphid is a more efficient vector of SrMV than sugarcane aphid, the importance of a vector in field conditions is decided based on its propensity, which measures both vector efficiency and abundance (Irwin and Ruesink 1986). Moreover, non-persistent transmission of viruses is best accomplished by non-host aphid species like *M. persicae*, which are known to be capable of causing severe mosaic epidemics in sugarcane. In addition to that, the incidence and increase of virus disease also depends on the number of migrating aphid vectors passing through the landscape. Therefore, the ability of *M. persicae* to cause SrMV disease epidemiology on sorghum fields should not be underestimated. In our feeding behavior studies using EPG, we found that *M. persicae* and *M. sacchari* differed in stylet penetration behaviors on sorghum

regardless of the infection status of the host. On infected hosts, *M. persicae* had higher numbers of probes within 30 minutes of experimental duration as compared to *M. sacchari.* Numbers of brief and frequent probes are an important characteristic in virus transmission by aphids, especially for non-persistently transmitted viruses (Powell 1991, Collar and Fereres 1998). Moreover, intracellular punctures by the stylet, associated with potential drop in EPG, are necessary for both acquisition and inoculation of non-persistent viruses (Powell 1991). In our experiment, we found that *M. persicae* required less time to produce intracellular punctures (i.e. potential drop) than *M. sacchari* which can play a significant role in virus transmission, especially during inoculation of non-persistently transmitted viruses which are short lived. Also, the frequency of potential drop has been reported to determine the transmission efficiency of a given aphid species (Powell et al. 1992, Collar et al. 1997). However, no significant differences in the frequency of potential drop were observed among two aphid species in our study. *M. persicae* produced a longer duration of pd than *M. sacchari* on SrMV-infected host. Mean duration of potential drop subphase II-1 and II-3, associated respectively with inoculation and acquisition of non-persistently transmitted viruses, and number of archlets in subphase II-3 of potential drops were significantly higher for *M. persicae* than *M. sacchari*. Consistent with our results, Collar and Fereres (1998) had earlier reported that longer II-3 subphase and presence of archlets were positively correlated with increased transmission efficiency of PVY by different aphid species in pepper (*Capsicum annuum* L.). However, Symmes et al. (2008) reported that shorter duration of pd was significantly related to successful acquisition of *Zucchini yellow mosaic virus* by *M. persicae* in melon (*Cucurbita pepo* L.) which contrasts our results.

Some of the significantly different stylet penetration behaviors among two aphid species feeding on virus-infected hosts were also observed to differ significantly on non-infected host in

our studies. Significantly higher numbers of probes were recorded by *M. persicae* than *M. sacchari* even on non-infected hosts. Similarly, longer durations of potential drops were produced by *M. persicae* than *M. sacchari.* A higher number of archlets were also produced by *M. persicae* than *M. sacchari* upon feeding on non-infected hosts. Therefore, it can be speculated that certain behavioral characteristics of some aphid species dispose themselves to be a better vector than others.

Upon comparing the probing behavior of *M. persicae* on virus infected and non-infected hosts, we found that significantly higher numbers of potential drops per probe as well as higher numbers of potential drops with archlets per probe were produced on infected hosts than on non-infected hosts. This behavior has a direct implication on virus transmission and spread as potential drop and archlets within potential drop are necessary waveforms for virus acquisition and inoculation especially for non-persistently transmitted viruses. Although this experiment was not designed to study the modification of vector behavior by plant virus, it is possible that SrMV might be altering the behavior of *M. persicae* through virus mediated changes in the host physiology and phenotype to enhance its spread. However, there were no significant differences in aforementioned behaviors of *M. sacchari*, a non-vector of SrMV.

Our findings demonstrate that transmission of SrMV differs among two aphid species; *M. sacchari* and *M. persicae*. The knowledge of transmission of SrMV from sorghum to sorghum by these two species is essential to precisely estimate the risk of transmission with respect to aphid species composition in sorghum fields. Our EPG results suggest that by virtue of producing a higher number of probes during feeding, a longer potential drop duration with longer subphases II-1 and II-3, higher number of archlets during subphase II-3, and quicker to reach the first potential drop; *M. persicae* possesses an ability to successfully transmit SrMV from sorghum to

sorghum while *M. sacchari* lacks it. Further virus transmission studies including other aphid species common in sorghum fields such as corn leaf aphid (*Rhopalosiphum maidis* Fitch), yellow sugarcane aphid (*Sipha flava* Forbes), or green bug (*Schizaphis graminum* Rodani) are required in order to fully understand the epidemiology of SrMV disease in sorghum fields.

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CHAPTER 4: THE PREFERENCE BEHAVIOR AND LIFE HISTORY TRAITS OF *MELANAPHIS SACCHARI* ON *SORGHUM MOSAIC VIRUS*-INFECTED AND NON-INFECTED SORGHUM

4.1. Introduction

Being obligate intracellular parasites and lacking their own molecular machinery, viruses require to infect a plant in order to replicate. Moreover, since plants are sessile, plant viruses need to employ strategies in order to move from one plant to another for dispersal in the landscape. The majority of plant viruses causing disease in agricultural crops depend on biotic vectors for transmission (Whitfield et al. 2015). Insects are the largest group of plant virus vectors which are comprised of aphids, thrips, leafhoppers, plant hoppers and whiteflies (Bragard et al. 2013). However, not all insects act as vectors for all plant virus-host pathosystems. Host selection by the vector is an important, complex process that involves different stimuli and responses (Fereres and Moreno 2009). The host selection process, especially for aphids, has been described in these steps: 1) visual and chemical attraction to a host plant, 2) alighting and initial assessment of the plant surface 3) probing on epidermis and mesophyll tissue, 4) stylet pathway activity, 5) sieve element puncture and salivation, and 6) phloem acceptance and sustained ingestion (Powell et al. 2006). The plant may be rejected by the insect at any of these stages after the evaluation of physical and/or chemical cues (Powell et al. 2006).

Physiological status of a plant can influence each of these steps, and because infection by virus alters the physiology of the plant, it can also change insect host selection process (Castle et al. 1998, Mauck et al. 2010, Dader et al. 2017). The first step in the host recognition process, attraction to a host plant, is an important initial event in the sequence with greater implications for virus transmission by vectors. Even though aphids have the tendency to land on and probe non-hosts, which is an integral part of the epidemiology of many aphid transmitted plant virus

diseases, the preference for a particular host before and after acquiring a virus plays a role in altering plant virus spread in the crop ecosystem (Blua and Perring 1992, Eigenbrode et al. 2002, Powell et al. 2006, Ingwell et al 2012, Rajabaskar et al. 2014). For example, some plant viruses have been reported to induce changes in host plants that cause potential aphid vectors to preferentially settle on virus infected plants (Castle et al. 1998, Eigenbrode et al. 2002, Jimenez-Martinez et al. 2004, Srinivasan et al. 2006, Alvarez et al. 2007, Mauck et al. 2010). In other cases, virus infected plants that are inferior hosts to the vectors seem to deter them (Blua and Perring 1992).

Most of the studies delineating attraction and feeding preferences of vectors, however, have focused on persistently and semi-persistently transmitted viruses while studies focusing on non-persistently transmitted viruses are limited. Mauck et al. (2010) reported that *Cucumber mosaic virus* (CMV), a non-persistently transmitted virus, infected squash (*Cucurbita pepo* L.) was initially attractive to *Aphis gossypii* Glover and *Myzus persicae* (Sulzer) due to the volatile organic compounds (VOCs) emitted by the infected squash plants. Earlier, Eckel (1990) had shown that aphids were more attracted to *Tobacco etch virus* infected tobacco plants (*Nicotiana tabacum* L.) than non-infected plants. Carmo-Sousa et al. (2014) reported that winged *A. gossypii* were attracted initially to CMV-infected cucumber plants (*Cucumis sativus* L.) but dispersed later to mock-inoculated plants under free choice assays.

Preference for virus infected plants by aphids has been ascribed to the yellowing of infected plant tissue, a visually more attractive coloration for aphids (Ajayi and Dewar 1983, Eckel and Lampert 1996). Virus induced changes in plant quality like amino acid content in phloem (Ajayi 1986, Blua et al. 1994) or soluble carbohydrate in leaves (Fereres et al. 1990) might influence behavioral responses of potential vectors but only after sap sampling. Moreover,

VOCs released by virus infected plants also influence the host selection behavior of aphids (Eigenbrode et al. 2002, Jimenez-Martinez et al. 2004, Mauck et al. 2010).

Once an aphid alights on the plant surface, a complex process involving different stimuli and responses ensues during its decision to colonize a plant (Powell et al. 2006). The aphid makes successive superficial probes and uses gustatory cues to differentiate between hosts and non-host plants. The plants infected with viruses induce changes in plant tissues which might elicit different results such as a combination of preferential settling and enhanced vector performance on infected hosts, neutral effects, or rapid dispersal and reduced vector performance on infected host depending upon different virus-vector-host combinations (reviewed in Eigenbrode et al. 2018). Generally, vectors feeding on persistently transmitted virus infected hosts have been reported to be positively affected and have a greater nymphal survival, adult fecundity, or increased growth rate (Fereres et al. 1989, Castle and Berger 1993, Srinivasan and Alvarez 2008) while vectors feeding on hosts infected by non-persistently transmitted viruses are found to be negatively affected due to poor quality of the hosts and disperse rapidly from the host instead of settling and sustained feeding on the host (Mauck et al. 2010). Semi-persistently transmitted viruses are alike in their effects on vector performance and behavior to persistently transmitted virus (Eigenbrode et al. 2018). It seems beneficial for persistently transmitted viruses to increase fitness of the aphids by increasing quality of the hosts as a strategy to lengthen feeding and ensure virus acquisition while non-persistently transmitted viruses would benefit from rapid dispersal of the aphids following brief probing/feeding as they are short-lived. However, nymphs of *M. persicae* were reported to have matured faster, and adults weighed more and produced more off springs on *Turnip mosaic virus* (TuMV) infected Chinese cabbage (*Brassica rapa* L.) (Hodgson 1981). Similarly, increased fitness of *M. persicae* was also detected

on TuMV-infected tobacco (*Nicotiana tabacum* L.). Moreover, aphid population parameters have also been reported to be affected by the nature of the infected hosts, depending upon whether the host is infected singly or mixed-infected. Wosula et al. (2013a) reported a significantly higher intrinsic rate of increase and the net reproductive rate in *M. persicae* reared on mixed virus infected sweet potato plants (infected by *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato virus G* (SPVG), and *Sweet potato virus 2* (SPV2)) compared to SPFMV-infected or non-infected plants. Some neutral effects have also been reported in few of the cases. CMV (P1 isolate)-infected pepper had neutral effects on *M. persicae* population as compared to population on healthy pepper plants (Mauck et al. 2014). Similarly, no significant differences were observed on development of thrips (*Frankliniella occidentalis* Pergande) upon feeding on Tomato spotted wilt virus-infected and non-infected *Datura stramonium* L. plants (Wijkamp et al. 1996).

The effect of plant viruses on the preference behavior and development of their vectors, therefore, is not uniform and varies depending upon the mode of transmission of virus. Moreover, very little research has focused on how herbivores and plant pathogens interact on a shared host-plant. Since *M. sacchari* populations are active in sorghum and rapidly expanding throughout the sorghum producing regions of the US, it is likely that aphids might encounter previously SrMV-infected plants and the information regarding how *M. sacchari* responds to virus infected sorghum is critically lacking. Here, we designed experiments to understand the preference behavior of two aphid species; *M. persicae*, a proven vector of *Sorghum mosaic virus* (SrMV), and *M. sacchari*, a non-vector of SrMV in our studies (chapter 3, this dissertation), on SrMV-infected and sham-inoculated sorghum. Sorghum is a host for *M. sacchari* while a non-host for *M. persicae*. The effects of SrMV on life history traits of *M. sacchari* were also studied.

4.2. Materials and Methods

4.2.1. Virus source and maintenance

Sorghum mosaic virus (SrMV-H) infected sorghum cv. Rio, confirmed through RT-PCR (Reverse Transcription- Polymerase Chain Reaction) test in Sugarcane Pathology Laboratory (Department of Plant Pathology and Crop Physiology, LSU, under the direction of Dr. J. Hoy), was obtained from the Sugarcane Pathology Laboratory and was maintained in the green house through mechanical inoculation. For mechanical inoculation, symptomatic leaf tissues were ground in extraction buffer (0.01 M phosphate buffer with pH 7.0 and 1.0% sodium sulphite) at the ratio of 1:2 (weight by volume) using a mortar and pestle and were applied with an absorbent cotton to healthy leaves of plants at the 3-4 leaf stage, previously dusted with 400 mesh carborundum powder (Fisher Scientific, Hampton, NH). After inoculation, plants were allowed to grow, symptoms were observed, and tissue were tested with DAS-ELISA (Double Antibody Sandwich- Enzyme Linked Immunosorbent Assay) as per manufacturer's protocols (Kit No. DEIAPV254, Creative Diagnostics, Shirley, NY, USA). Non-symptomatic plants as well as plants testing negative through ELISA were discarded.

4.2.2. Aphid colonies

Sugarcane aphids used in the experiments were obtained from LSU-SCA14 colony, founded from a single apterous *M. sacchari* collected from sorghum field at the Louisiana State Agricultural Center, Dean Lee Research Station, Alexandria, LA in July 2014 by Dr. J. A. Davis. The colony was maintained on Pioneer 85G85, a sorghum hybrid resistant to greenbug (Pioneer Hi-Bred International, Inc., Johnston, IA), grown in plastic pots of 10 cm diameter containing sterile potting mix (Miracle-Gro Organic Choice Garden Soil, Marysville, OH) and 5g Osmocote (14:14:14), a slow releasing fertilizer (The Scotts Company, Marysville, OH). The plants were

maintained in growth chambers (Percival Scientific, Perry, IA) at 25 ± 2 °C, 50 ± 5% RH and a photoperiod of 14:10 (L: D). The colony was maintained in screened cages under laboratory conditions at room temperature (20-22 °C) and a 14:10 (L: D) photoperiod.

Green peach aphids used in the experiment were obtained from a colony established from a single apterous *M. persicae* collected from eggplant (*Solanum melongena* L.) in 2009. The colony was maintained in screened cages (30 x 30 x 30 cm, constructed using Plexiglass plastic sheet and nylon mesh fabric) and reared on mustard (*Brassica juncea* L.) cv. Tendergreen (W. Atlee Burpee and Co., Warminster, PA), under laboratory conditions at room temperature (20-22 °C) and a 14:10 (L: D) photoperiod. Mustard plants were planted in plastic pots 10 cm in diameter (Dillen Products, Middlefield, OH) containing sterile potting mix (Miracle-Gro Organic Choice Garden Soil, Marysville, OH) and 5g Osmocote (14:14:14) (The Scotts Company, Marysville, OH) and grown in growth chambers (Percival Scientific, Perry, IA) maintained at 25 ± 2 °C, 50 ± 5% RH and a photoperiod of 14:10 (L: D). A cohort of 5 to 10 aphids was placed using a paint brush on fresh plants every 2 to 3 weeks to establish a new colony.

4.2.3. Host plants

Sorghum cv. Rio was used as the host plant for both preference and feeding bioassays. Sorghum seeds were sown in plastic pots 10 cm in diameter (Dillen Products, Middlefield, OH) containing sterile potting mix (Miracle-Gro Organic Choice Garden Soil, Marysville, OH) and 5g Osmocote (14:14:14) (The Scotts Company, Marysville, OH) and maintained in the greenhouse at 22 to 28 °C with varying RH. SrMV infected host plants were maintained by mechanical inoculation at 3-4 leaf stage. Non-infected host plants (control) used for the experiments were inoculated with virus extraction buffer only (sham-inoculated). Plants were grown inside tent-like cages 61 x 61 x 61 cm in dimension (BugDorms (Catalog No. 1462W), BioQuip Products, Inc., USA) in order

to ensure no cross transmission. Plants were carried to the laboratory for preference bioassay experiments after 3 weeks of mechanical inoculation and after allowing sufficient time for the virus infected plants to develop characteristic mosaic symptoms.

4.2.4. Dual-choice test bioassays

Host plant selection preference of apterous non-viruliferous *M. sacchari* and *M. persicae* were examined using a dual choice bioassay arena. The arena consisted of a petri dish (15 x 1 cm diameter) (Fisherbrand Petri Dishes, Thermo Fisher Scientific Inc., Pittsburgh, PA) with two notches, each 7cm long and 2 cm wide, cut across the diameter to insert plant leaves from two but opposite directions (Fig 4.1). A gap 1-cm long is left at the center between two notches so that the leaves do not touch each other. The notch is open at the top so that aphids can move freely and feed on the preferred leaf surface while the bottom is closed with no see-um netting fabric for aeration. A small-hole (approximately 0.5 cm in diameter) was drilled towards the edge of the plate, equidistant from two notches, in order to insert and hold a 2 ml micro-centrifuge tube that contained and released the aphids at the beginning of the experiment.

Two plants, a virus infected and a sham-inoculated plant (approximately 4-5 weeks old) were placed on two sides of the arena. A single upper leaf from both plants were inserted into the arena through respective notches on each side. Thirty adult apterous aphids were removed from their respective colonies with the help of a fine camel's hair brush and placed in a 2 ml micro-centrifuge tube. After a starvation period of 2 h inside the micro-centrifuge tube, the tube was opened in order to let the aphid climb to the choice arena and make a preference decision. The arena was covered with the lid and sealed with parafilm (Parafilm, Pechiney Plastic Packaging, Menasha, WI). The aphids were observed after 0.5 h, 1 h, 2 h, 4 h, and 24 h and the number of aphids observed in each leaf segment were counted during each observation period. The earlier

observations were aimed at examining the initial preference while later observations were made in order to understand the overall movement of aphids across the treatments. The experiment was conducted in a windowless room with diffuse artificial lighting under laboratory conditions at room temperature (20-22 °C) and a 14:10 (L: D) photoperiod. The positions of plants were varied during each replication to avoid continuous placing of one treatment on one side of the arena. The experiment was replicated 20 times and the sample sizes were consistent with previously published studies including similar experiments (Mauck et al. 2010, Carmo-Sousa et al. 2014). The proportion of aphids responding to either treatments were compared using a generalized linear mixed model assuming a binomial distribution with a logit link function in SAS (Proc Glimmix, SAS Institute version 9.4, Cary, NC) as described in previous published studies (Ingwell et al. 2012, Rajabaskar et al. 2014). The time variable examined observations made at different intervals after release using a repeated measures design. Treatment means were separated by the Tukey-HSD test ($\alpha = 0.05$). Aphids not located on either plant leaf in an arena were considered non-responsive and excluded from the analysis.

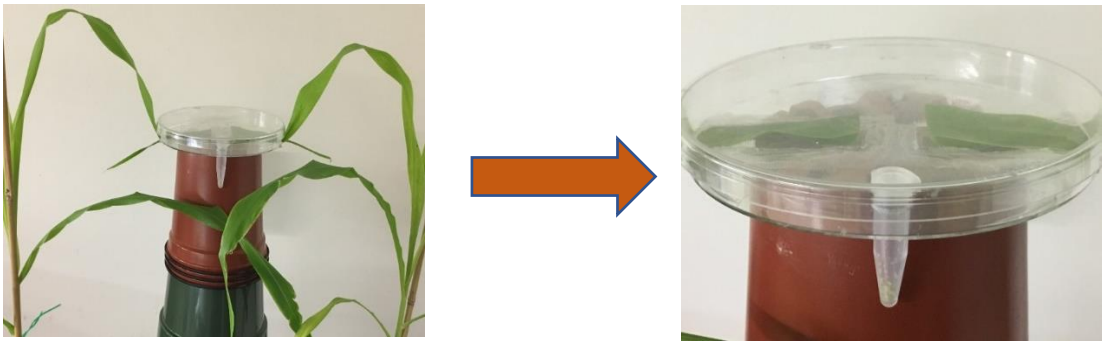

Fig. 4.1. Showing the experimental setup of the dual choice bioassay (left) and a close-up of the arena (right).

4.2.5. Life table studies

The life table studies of *M. sacchari* on two different hosts, SrMV-infected and sham-inoculated sorghum cv. Rio was conducted and aphid life history traits were compared on the

host plants using excised leaves. The leaf sections approximately 3 cm x 2 cm (length x breadth) in dimension were excised from the host plants grown and maintained in the green house as described above and placed in 30 ml Solo cups (Dart Container Corporation, Mason, MI). The cups were filled previously with 12-15 ml agarose solution (0.1% w/v) (RM3001-500G Agar Powder Extra Pure, HiMedia, Einhausen, Germany) as described by De Souza (2018). Leaf sections from young leaves (and symptomatic in case of infected host treatment) were placed with abaxial surface upward on top of the agarose solution. Agarose solution avoided dehydration of the leaves and prevented aphids from escaping the leaf surface (Sampaio et al. 2001). The leaf sections were replaced every 3-4 days. The experiment was conducted at a constant temperature of 25 °C in climate regulated chambers (Model I-41VL, Percival scientific, Perry, IA). A single apterous adult aphid was placed on the leaf section with the help of a fine hair paint brush and allowed to larviposit for 24 h. After 24 h, the adult aphid along with all nymphs but one were removed, leaving only one nymph in the cup. All single first instar nymphs were the cohort for that treatment for the duration of the experiment. There were 45 nymphs per cohort and the experiment was repeated three times for both treatments. The cohort was examined every 24 h for deaths, change of instars, and number of nymphs produced per day until death for all individuals. Life table parameters were calculated for each host plant as described by Birch (1948). Age-specific survival (l_x) and fecundity (m_x) of aphids were calculated for each host. Net reproductive rate, R_0, defined as the product of age-specific survival and age-specific fecundity was calculated using the formula $R_0 = \sum l_x m_x$, where l_x is the proportion of live females on a given day, and m_x is the mean number of births by female on that day. The intrinsic rate of increase, r_m, was calculated as, $\sum e^{-r_m} l_x m_x = 1$. Finite rate of increase was calculated as, $\lambda = e^{r_m}$. Mean generation time was calculated as,

$T_G = \ln R_0/r_m$, and doubling time was calculated as, DT = ln (2)/r_m. Jackknifing procedure was used to estimate r_m standard error. Jackknifing is based on recombining the original data, calculating the pseudo-values for each recombined original data and estimating the mean value and standard error of r_m from the frequency distribution of pseudo-values (Meyers et al. 1986). Pseudo-values of r_m were used to estimate the values for net reproductive rate, finite rate of increase, mean generation time and population doubling time and these variables were analyzed by one-way analysis of variance in SAS (Proc Glimmix, SAS Institute version 9.4, Cary, NC). Treatment means were separated by the Tukey-HSD test ($\alpha = 0.05$). The biological variables (days to reproductive adult, reproductive period, longevity, progeny per female, and progeny per female per day) were analyzed using a randomized block design. Proc Glimmix procedures in SAS 9.4 (SAS institute Inc., 2013, Cary, NC) were used for all the datasets and Tukey-HSD at 0.05% significance allowed us to compare the least square means differences among treatments for each variable. Age-specific survivorship and fecundity graphs were plotted using Excel (Microsoft Excel Ver. 3, 2013).

4.3. Results

4.3.1. Dual-choice test bioassays

Aphid preference behavior experiments in the dual-choice arena showed that apterous *M. sacchari* preferred SrMV-infected sorghum plants at early stages (0.5 h) ($F = 8.02$, $P = 0.0073$). However, no significant differences were observed in mean proportion of aphids among treatments during later stages (1 h, 2 h, 4 h and 24 h after release) ($P > 0.05$). The proportion of aphids observed on SrMV-infected leaf segments were constantly higher than on sham-inoculated leaf segment although differences were not significant after first observation (0.5 h) ($P > 0.05$) (Fig. 4.2). On the other hand, *M. persicae* apterous adults didn't show any particular

preference among treatments in our studies (P > 0.05). Comparatively lower proportions of *M. persicae* adults were observed in either of the treatments as compared to *M. sacchari* although the experiment was not designed to test this difference. The proportion of *M. persicae* observed on sham-inoculated leaf segment didn't vary throughout the observation periods (Fig. 4.3). However, *M. persicae* tended to prefer sham-inoculated leaf segment at earlier observations (0.5 h), although no significant differences among treatments were observed. The proportion of *M. persicae* observed was greater on SrMV-infected leaf segment at 1 h and 2 h mark than on sham-inoculated leaf, but the difference was not statistically significant ($P > 0.05$). Aphids tended to move away from the SrMV-infected leaf segment after 2 h of release, as illustrated by the decreased proportion of aphids observed on the infected leaf segment (Fig 4.3).

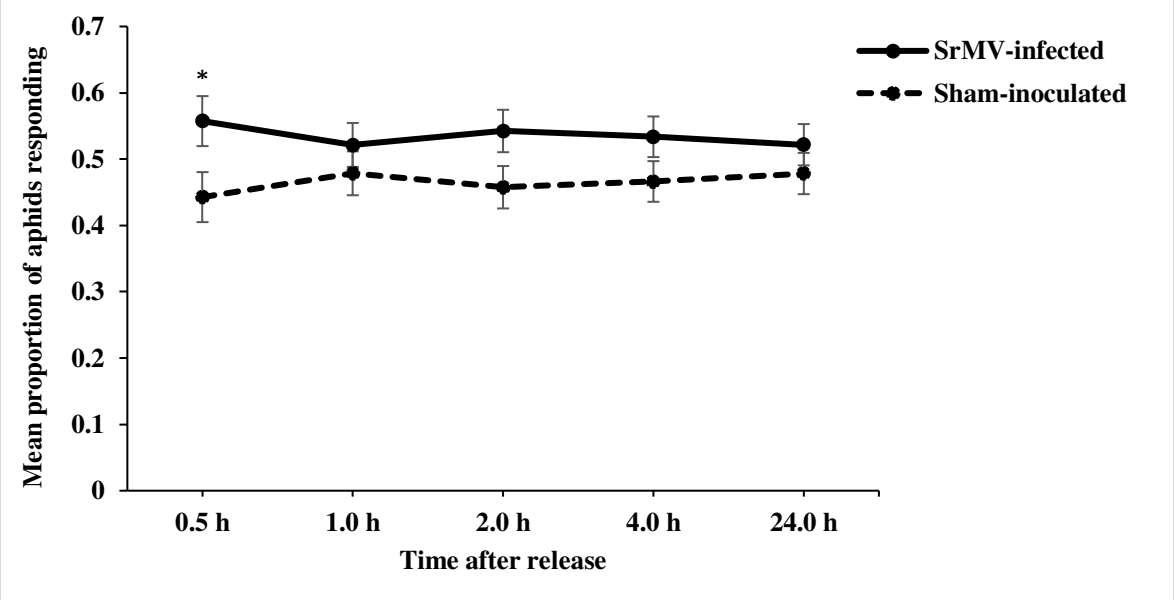

Fig. 4.2. Mean proportion of responding apterous *M. sacchari* adults observed on SrMV-infected and sham-inoculated sorghum plants under dual-choice assays at 0.5 h, 1 h, 2 h, 4 h and 24 h after aphid release. *Significant differences according to Tukey-HSD test (P < 0.05).

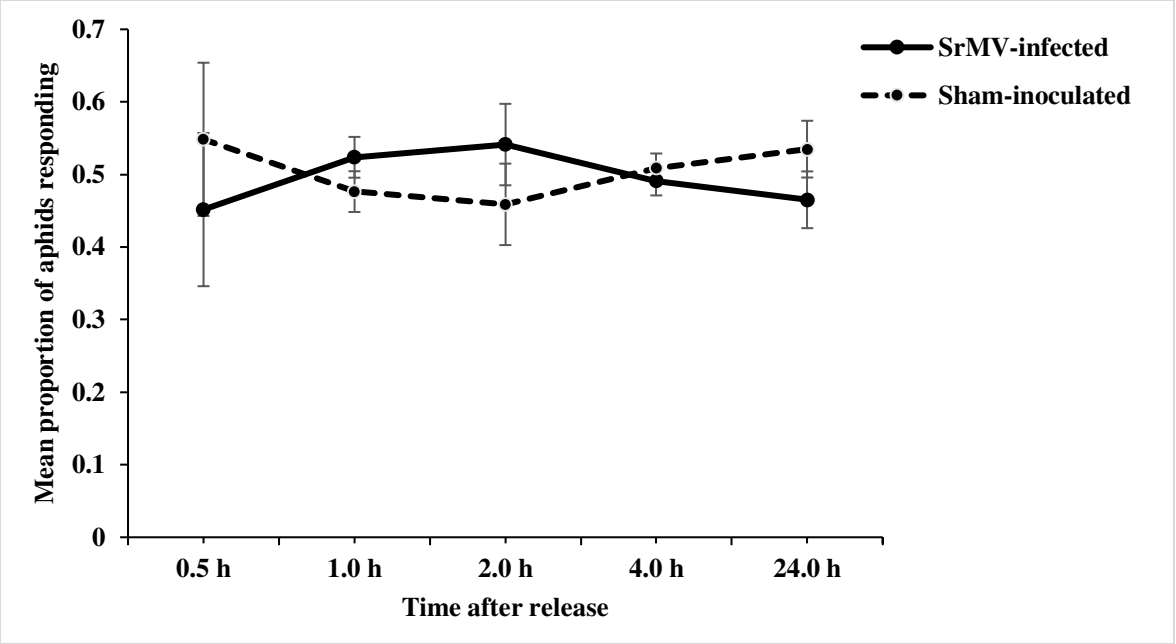

Fig. 4.3. Mean proportion of apterous *M. persicae* adults present on SrMV-infected and sham-inoculated sorghum plants under dual-choice assays at 0.5 h, 1 h, 2 h, 4 h and 24 h after aphid release. No significant differences among treatments according to Tukey-HSD test (P > 0.05).

4.3.2. Life table analysis

M. sacchari cohorts evaluated in the laboratory through feeding bioassays on SrMV-infected and sham-inoculated sorghum showed statistically significant differences for some of the biological variables (Table 4.1). No significant differences were found in days to reproductive adults. Similarly, differences were not detected in reproductive period (the amount of time in which the female remained reproductively active). However, aphids fed on sham-inoculated sorghum leaf tissues had a greater longevity (average lifespan from day one until death) than those fed on SrMV-infected leaf tissues ($F = 4.27$, $P = 0.04$) (Table 4.1). Similarly, statistically significant differences were observed in two reproductive variables, progeny per female ($F = 5.67$, $P = 0.031$) and progeny per female per day ($F = 12.45$, $P = 0.0005$), where aphids performed better upon feeding on sham-inoculated leaf tissue.

Among the population parameters evaluated, intrinsic rate of increase (r_m) was higher in the sham-inoculated treatment as compared to SrMV-infected treatment. There were no significant differences in other parameters calculated (Table 4.1).

Age-specific survival rate (l_x) was higher throughout the duration of experiment on sham-inoculated sorghum as compared to SrMV-infected sorghum (Fig. 4.4). Similarly, age-specific fertility rate was also higher on sham-inoculated sorghum (Fig. 4.5).

Table 4.1. Life-table parameters (mean ± SE) of *M. sacchari* on SrMV-infected and sham-inoculated sorghum. Values followed by different letters in a row are significantly different by the Tukey-HSD test ($\alpha = 0.05$).

	Host plant	
Parameters	Sham-inoculated	SrMV-infected
Biological parameters		
Days to reproductive adult	6.8 ± 0.2 a	6.9 ± 0.2 a
Reproductive period	10.5 ± 0.5 a	10.8 ± 0.5 a
Longevity	18.7 ± 0.8 a	16.0 ± 0.8 b
Progeny per female	53.3 ± 2.4 a	46.7 ± 2.6 b
Progeny per female per day (m_x)	4.9 ± 0.2 a	3.9 ± 0.2 b
Population parameters		
Intrinsic rate of increase (r_m)	0.393 ± 0.008 a	0.370 ± 0.010 b
Net reproductive rate (R_0)	38.1 ± 10.2 a	27.9 ± 6.8 a
Finite rate of increase (λ)	1.48 ± 0.03 a	1.45 ± 0.07 a
Doubling time (D_T)	1.6 ± 0.1 a	1.7 ± 0.3 a
Mean generation time (G_T)	9.3 ± 0.5 a	8.9 ± 0.8 a

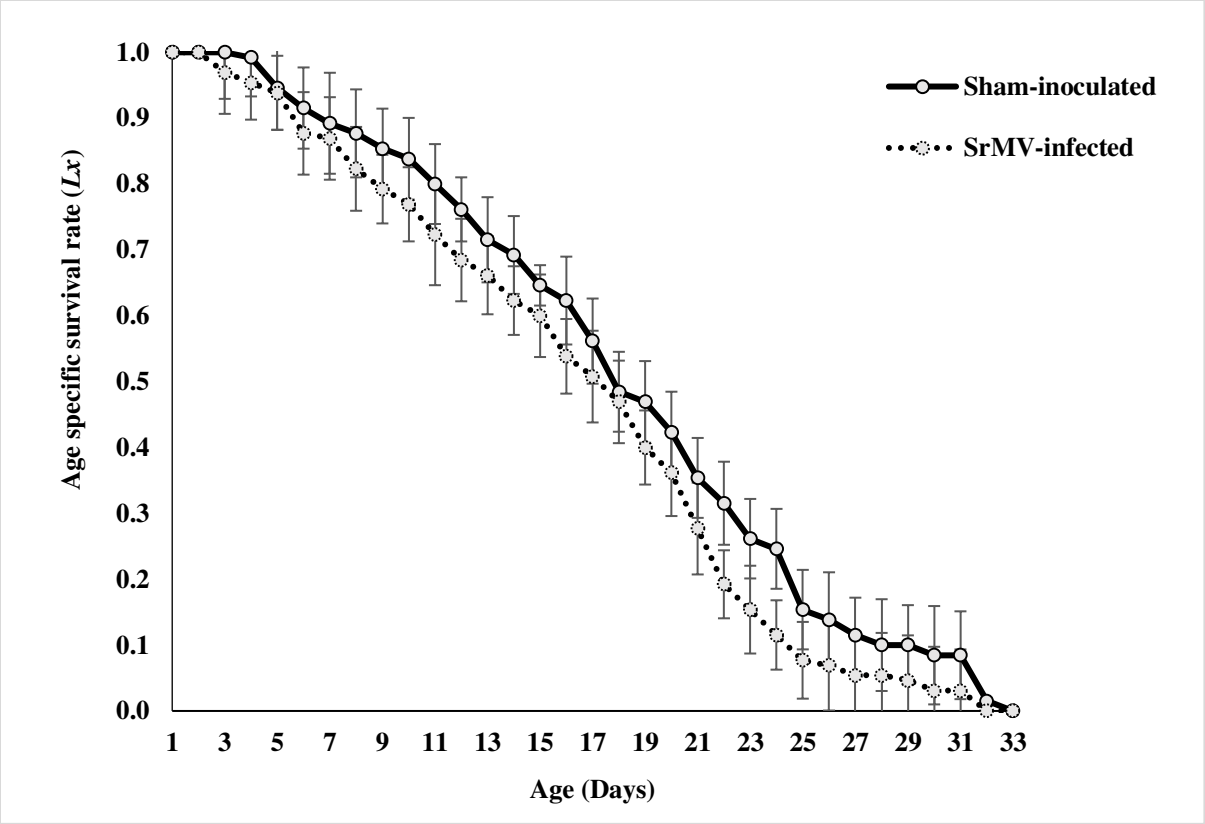

Fig. 4.4. Age-specific survivorship of *M. sacchari* on SrMV-infected and sham-inoculated sorghum.

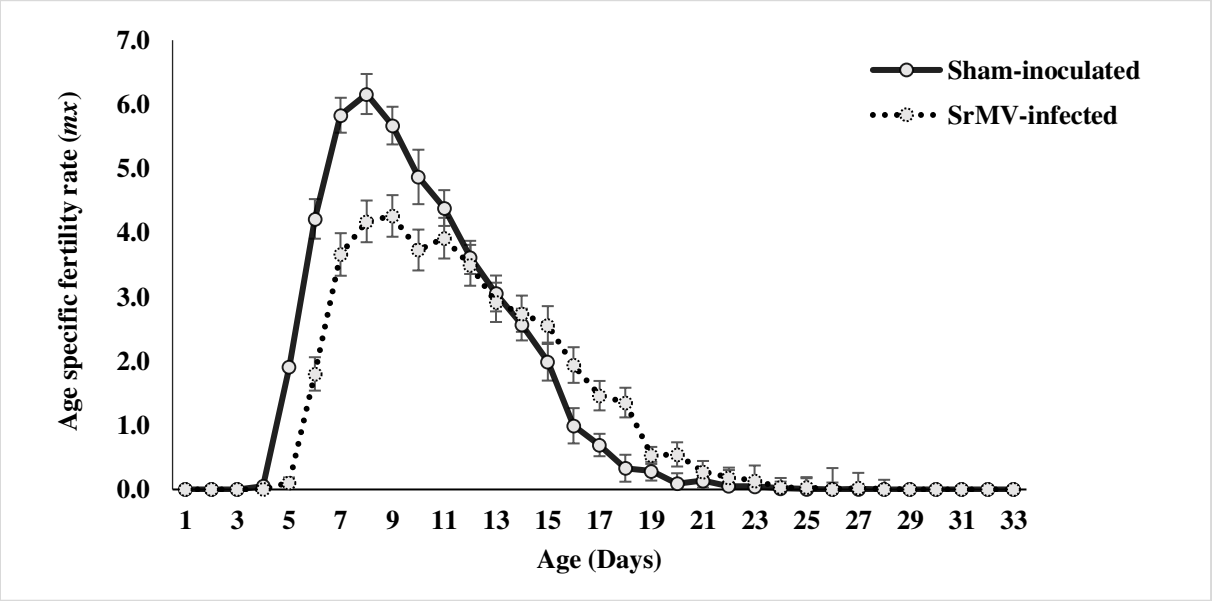

Fig. 4.5. Age-specific fertility rate of *M. sacchari* on SrMV-infected and sham-inoculated sorghum.

4.4. Discussion

The transmission and spread of plant viruses depend upon virus-vector relationship and this relationship can be regulated by the virus in order to acquire an adaptive advantage (Blua and Perring 1992). Plant viruses have been demonstrated to induce specific changes in plant volatiles emission and change the physiology and morphology of the plant (Bosque-Perez and Eigenbrode 2011, Mauck et al. 2012). In addition to that, nutritional conditions of the plant infected by the virus and altered plant defense mechanisms in infected plants might also modulate virus-vector relationships (Blua et al. 1994, Mauck et al. 2010).

The spread of aphid-transmitted viruses, as indicated by numerous models, is enhanced if vectors favorably settle and feed on plants infected with virus as compared to non-infected plants (Sisterson 2008). Previous studies have demonstrated that both visual and olfactory signals emitted by the plant can change alter aphid behavior in discriminating infected or non-infected plants (Eigenbrode et al. 2002, Medina-Ortega et al. 2009, Mauck et al. 2010). Generally, infected plants become more attractive to vectors across pathosystems but the consistency of the effects is highly variable among pathogens and pathosystems (Eigenbrode et al. 2018). Vector behavior studies, however, have generally concentrated on pathosystems containing persistently transmitted viruses. Regardless, for a consistent acceleration of virus spread, the vectors settling on infected plants need to feed on them until they have acquired the virus and move to non-infected plants (Roosien et al. 2013).

In our studies with SrMV-sorghum pathosystem and its interaction with a potential vector, *M. persicae*, we found that apterous *M. persicae* didn't show a particular preference for either virus infected or sham inoculated leaves. We found no significant differences in the proportion of aphids observed at different time intervals after release among virus infected or

sham-inoculated leaf segments. Though the differences were statistically non-significant, a decrease in proportion of aphids on virus infected leaf segments was observed at later time periods, even reaching lower than aphids observed on sham-inoculated leaf segments towards the end of the experimental period (Fig. 4.3). Although we did not test the presence of virus in aphids during the experiment, the decreasing trend might suggest a proposed generalization of rapid emigration behavior of aphids carrying non-persistently transmitted viruses from infected source (Mauck et al. 2012). It is worth mentioning that even a small change in vector activity can have large effects on the spread of non-persistently transmitted viruses (Madden et al. 2000). Non-preference to a particular host (infected vs non-infected) by a vector in our study, however, is consistent with previous results documented by Fereres et al. (1999) on two non-persistently transmitted viruses, PVY and *Soybean mosaic virus* (SMV), where no preference among virus infected and non-infected soybean (*Glycine max* L.) and pepper (*Capsicum annuum* L.) plants by *M. persicae* and *Rhopalosiphum maidis* (Fitch) were reported. On the contrary, *M. sacchari*, a non-vector of SrMV (Chapter 3, this dissertation), interestingly, preferred SrMV infected sorghum than sham-inoculated in the earlier stages of our experiment (0.5 h after release). The mean proportion of aphids observed in later time points however, were not statistically significant among treatments. Prior studies suggested that preference of aphids to different host plant species might also affect their host selection behavior (Srinivasan et al. 2006). The host plant used in our studies, sorghum, is a non-host of *M. persicae* whereas it is a much preferred host of *M. sacchari.* This was evident by failed attempts of life table assays we conducted for *M. persicae* on sorghum where the cohorts couldn't survive more than 1-2 days (data not shown). Moreover, the proportion of *M. persicae* observed in either of the treatment leaves in choice bioassay was very low as compared to the experiment on *M. sacchari* choice bioassay. The

proportion of *M. sacchari* aphids observed on SrMV-infected leaves didn't fluctuate greatly and remained consistently higher than on sham-inoculated leaves throughout the experimental duration (Fig. 4.1). These observations suggest that apterous *M. sacchari* behavior might have been influenced by virus initially and were attracted to virus-infected leaves. However, there is no evidence to suggest that the behavior of *M. sacchari* was affected upon feeding on virus infected leaves as the proportion of aphids observed on infected leaves remained consistent and didn't fluctuate rapidly throughout the experimental duration. It is not surprising for virus to have no effects on *M. sacchari* behavior upon feeding on virus infected leaves since it is a non-vector of SrMV.

Besides the direct manipulation of the vector behavior before and after feeding, some viruses are also known to change the appropriateness of host plants for aphid vectors and thus affect the growth and development of their vector herbivores. The majority of the published report indicates that virus-infected plants are better hosts than virus-free plants in terms of vector growth rates, reproduction, and longevity (Fereres et al. 1989, Castle and Berger 1993, Blua et al. 1994, Jimenez-Martinez et al. 2004, Srinivasan et al. 2008). However, some negative effects on vectors have also been reported (McIntyre et al. 1981, Donaldson and Gratton 2007, Jimenez-Martinez et al. 2009). In our study, we examined the potential interaction between a plant virus and an insect pest sharing the same host plant species through feeding bioassay experiment in the laboratory. We found that SrMV negatively affected the longevity and population growth rates of *M. sacchari.* Intrinsic rate of increase was negatively affected on *M. sacchari* cohorts fed on SrMV-infected leaf tissue. Age-specific survival rate was consistently higher on aphids fed on sham-inoculated leaf tissue as compared to SrMV-infected ones (Fig. 4.3). Here, we report the first evidence of effects of plant viruses on the growth, development, and reproduction of *M.*

sacchari in laboratory conditions. In addition, our findings will also help to add some insights into a limited pool of information regarding the effects of non-persistently transmitted viruses on aphid herbivores. Our findings have implications both for aphid populations in the sorghum fields and for patterns of transmission of SrMV. Even though sorghum is a non-host for *M. persicae* and their populations are rarely seen on sorghum fields, non-persistent transmission is best accomplished by non-host aphid species like *M. persicae*, which are known to be capable of causing severe mosaic epidemics in sugarcane. Moreover, the incidence and increase of virus disease also depends on the number of migrating aphid vectors passing through the landscape. Therefore, the ability of *M. persicae* to cause SrMV disease epidemiology in sorghum fields should not be underestimated. However, the neutral preference of *M. persicae* in our experiment (SrMV-infected versus sham-inoculated leaf), in contrast to a general observation for non-persistently transmitted viruses, bodes well for low rates of increase in SrMV-disease incidence on sorghum fields.

The findings of this study contribute toward elucidating the complex interrelationships that occur among sorghum, its associated virus (SrMV), and *M. sacchari*. Even though mechanisms for decreased population growth rate is unclear, life table analyses indicate that decreased survivorship on infected sorghum may result in decreased aphid performance in the field. Lower population growth rates could lengthen the duration of time aphid populations in the field are below economic threshold thus reducing the extra losses incurred by aphids. In addition, the slower population growth would enable natural enemies to efficiently minimize aphid populations. Besides *M. sacchari*, many other aphid species such as corn leaf aphid (*Rhopalosiphum maidis* Fitch), yellow sugarcane aphid (*Sipha flava* Forbes), and green bug (*Schizaphis graminum* Rodani) frequently visit sorghum fields in the US. Understanding the

efficiency of all these aphid species to transmit SrMV in sorghum and the effects of this virus on their behavior and performance is crucial in predicting the incidence and severity of SrMV-disease occurrence on sorghum fields. Moreover, an understanding of mechanisms of direct impacts of plant viruses on aphids may initiate novel avenues for the management of aphid populations in sorghum fields.

4.5. References

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CHAPTER 5: IMPACTS OF *CUCUMBER MOSAIC VIRUS* AND *SUNN-HEMP MOSAIC VIRUS* ON FEEDING, GROWTH AND OVIPOSITION PREFERENCE OF TWO NON-VECTOR HERBIVORES

5.1. Introduction

Plant viruses are rampant in both natural and artificial ecosystems and infections by plant viruses alter the phenotypic as well as the physiological characteristics of their host plants (Blua et al. 1994, Jeger et al. 2004, Agrawal et al. 2006). These changes ultimately affect vector behavior by attracting non-viruliferous vectors to virus infected plants for virus acquisition and spread (Eigenbrode et al. 2002, Ingwell et al. 2012) and vector population dynamics by supporting vector population growth (Ajayi et al. 1983, Alvarez et al. 2007). Virus-vector-host interactions can be positive, negative, or neutral. Plant viruses have been known to increase the attractiveness of their host plants by changing their physical and chemical appearance (Eigenbrode et al. 2002, Mauck et al. 2010). Moreover, it has also been shown that feeding preferences of insect vectors, after virus acquisition, can be altered (Stafford et al. 2011, Ingwell et al. 2012, Shrestha et al. 2012, Rajabaskar et al. 2014). Rajabaskar et al. (2014) found that non-viruliferous *Myzus persicae* aphids preferred potato (*Solanum tuberosum* L.) plants infected with *Potato leafroll virus* whereas viruliferous aphids preferred uninfected plants. In addition, virus induced changes in host plants affect the fitness of arthropod vectors both positively (Fereres et al. 1989; Jimenez-Martinez et al. 2004; Maris et al., 2004; Srinivasan et al. 2008) and negatively (Blua and Perring 1992, Garcia et al. 2000; Stumpf and Kennedy 2005, Shrestha et al. 2012). Shrestha et al. (2012) demonstrated that fewer potentially viruliferous *Frankliniella fusca* (Hinds) eggs developed into adults and also required longer developmental time, insinuating a negative effect on fitness due to the presence of virus. No effects of virus induced host changes on vector fitness have been reported in some other instances (Wijkamp et al. 1996; Roca et al. 1997).

However, the interactions between plant viruses and non-vector arthropod herbivores have been poorly studied. Hare and Dodds (1987) reported an improvement in *Leptinotarsa decemlineata* (Say) survival on *Tobacco mosaic virus* (TMV) infected tomato. Similarly, the growth of *Spodoptera exigua* (Hubner) caterpillars increased on TMV-infected tomato (*Solanum lycopersicum* L.) plants as compared to control plants (Thaler et al. 2010). In one study, tomato plants infected by *Tomato mosaic virus* did not affect the feeding or oviposition preferences of corn earworm, *Helicoverpa armigera* (Hubner) (Lin et al. 2008). Spider mite survival and oviposition was enhanced by *Tomato spotted wilt virus* (TSWV) infection on pepper plants (Belliure et al. 2010). Mauck et al. (2010) reported that *Anasa tristis* (DeGeer), a non-vector herbivore, preferred to lay more eggs on healthy squash plants as compared to CMV infected plants. Alternatively, fungus gnat females preferred to oviposit on non-infected rather than *White clover mosaic virus* infected white clover plants, although fungus gnat larvae were only marginally affected by the virus (van Molken et al. 2012). Hence, the impact of virus infection of the host plant on the behavior and fitness of non-vector herbivores is difficult to predict at present.

Soybean looper, *Chrysodeixis includens* (Walker) [Lepidoptera: Noctuidae], is an important polyphagous pest and one of the most important and difficult pests to manage owing to their ability to defoliate rapidly (Herzog 1980, Mascarenhas and Boethel 1997, Moonga and Davis 2016)). Similarly, fall armyworm, *Spodoptera frugiperda* (Smith) [Lepidoptera: Noctuidae], is also an important crop pest which has a very wide host range including many grasses, cereal crops, vegetable crops and cotton (Pashley 1988, Walton and Luginbill 1916, Hinds and Dew 1951). Fall armyworm has been one of the primary pests of field corn in southern US historically (Pitre and Hogg 1983, Buntin 1986).

Cucumber mosaic virus (CMV) is the type member of genus *Cucumovirus* and family *Bromoviridae*, infecting more than 1200 plant species worldwide (Palukaitis et al. 1992). CMV is an important model for research owing to its easy mechanical transmissibility, strong virus accumulation in infected hosts, and the largest host range of any virus (Palukaitis et al. 1992). *Sunn-hemp mosaic virus* (ShMV) is a type member of genus *Tobamovirus* and family *Virgaviridae* and infects many crops in the Family *Leguminosae* (Boswell and Gibbs 1983). CMV and ShMV are both non-persistently transmitted by aphids and their effects on non-vector herbivores are not clearly understood. Both these viruses infect cowpea and cause mosaic symptoms, stunted growth, and eventual yield loss (Arogundade et al. 2009, Pio-Ribeiro et al. 1978). However, the impacts these viruses can incur on non-vector herbivores of cowpea are unknown. Therefore, greenhouse and laboratory experiments were conducted to determine the effects of *Cucumber mosaic virus* and *Sunn-hemp mosaic virus* infected cowpea on soybean looper and fall armyworm.

5.2. Materials and Methods

5.2.1. Insect colonies

The soybean looper colony used in this study was MR08 which was collected from soybean fields at the Macon Ridge Research Station (Winnsboro, LA) in 2008 (Brown, 2012). The colony was maintained by rearing larvae in 30 ml plastic cups (2-3 larvae/cup) on 10 ml artificial diet (Southland Products, Lake Village, AR). The rearing room was maintained at 28.5 °C, 50% R.H., and 14:10 (L: D) photoperiod. After pupation, pupae were reared in 3.8 L plastic containers (United States Plastic Corp, Lima, Ohio) with 25-30 g vermiculite (Sun Gro, Bellevue, WA). At the center of each containers, two 30 ml cups containing cotton wadding saturated in 10% honey solution were placed as supplementary diets for adults (Mascarenhas and

Boethel 2000). Paper towels cut in strips (8 x 20 cm) surrounded the inside of each containers and were used as oviposition sheets for the adults (Jensen et al. 1974). The oviposition sheets and honey solution were replaced every 2-3 days and egg sheets were kept in plastic bags until larval hatching (Chen 2018).

Fall armyworms used in this study were Bt-Susceptible biotype (genotype aabb) obtained from Corn Entomology Laboratory (Department of entomology, LSU under the directin of Dr. F. Huang). Genotype aabb was generated using an F2 screen of two-parent family lines of *S. frugiperda* derived from a field population collected from non-Bt maize fields in Franklin Parish, Louisiana in 2016 (Dr. Huang, personal communication). The larvae were reared on meridic diet (Ward's Stonefly Heliothis diet, Rochester, NY) in 30-ml plastic cups (Fill-Rite, Newark, NJ) until the pupal stage under room conditions and pupae were placed in 3.8-L paper containers for adult emergence, mating, and oviposition as described by Niu et al. (2013).

5.2.2. Virus source and maintenance

Inoculum for both the viruses, CMV and ShMV, were obtained from the Sweet Potato Pathology Laboratory (Department of Plant Pathology and Crop Physiology, LSU, under the direction of Dr. C. Clark). The viruses were obtained initially through infected seeds in cowpeas and were tested using Enzyme Linked Immunosorbent Assay (ELISA) (Dr. Clark, personal communication).

5.2.3. Test plants

Cowpea var. Quick Pick Pinkeye (Victory Seed Company, Molalla, OR) was used for the experiments. Seeds were planted in 10-cm-diameter plastic pots (Dillen Products, Middlefield, Ohio) containing sterile potting mix (Miracle-Gro Potting Mix, The Scotts Company, Marysville, OH) and 5g Osmocote (14:14:14) (The Scotts Company, Marysville, OH) was mixed into the

soil prior to planting of seeds as a nutrient supplement and the pots were watered as required. Plants were grown in a greenhouse at 27 ± 1 °C, 70 ± 10% RH, and a 14:10 (L:D) h photoperiod.

5.2.4. Mechanical inoculation of host plants

After a week of germination, plants were mechanically inoculated with respective viruses. For mechanical inoculation, plant tissues infected with viruses (either CMV or ShMV) were ground in extraction buffer (0.1 M phosphate buffer with pH 7.0 and 1.0% sodium sulphite) at the ratio of 1:2 (weight by volume) by using a mortar and pestle. Absorbent cotton dipped in virus extracted buffer solution was used to gently rub leaves of healthy test plants which were previously dusted with 400 mesh carborundum powder (Fisher Scientific, Hampton, NH). Test plants showing visual symptoms after 2-3 weeks of inoculation were used for the experiment while the non-symptomatic plants were discarded. For control treatment (sham-inoculated), carborundum dusted plant leaves were inoculated with extraction buffer solution alone. The plants were maintained in greenhouse at 22-28°C and a varying RH. In order to ensure no cross contamination occured, the plants were kept inside tent-like cages 61 x 61 x 61 cm in dimension (BugDorms, Catalog No. 1462W), BioQuip Products, Inc., USA).

5.2.5. Feeding bioassays

After two weeks, virus-infected and sham-inoculated leaves were brought to the laboratory from the green-house. Newly hatched neonates were obtained from their respective colonies and maintained in 30-ml Solo cups (Dart Container Corporation, Mason, MI) for 24 h in 10 ml soybean looper artificial diet (Southland Products, Lake Village, AR) for soybean looper and on meridic diet (Ward's Stonefly Heliothis diet, Rochester, NY) for fall armyworm in order to reduce initial mortality due to handling. After 24 h, neonates of soybean looper and fall armyworm were transferred to sterile petri-dishes (10 X 1.5 cm) (Fisherbrand Petri Dishes,

Thermo Fisher Scientific Inc., Pittsburgh, PA) containing a moistened filter paper (9.0 cm Grade 410 filter paper, VWR International, Suwanee, GA, USA) with the help of a fine hair paint brush. Neonates were allowed to feed on the leaf cores; virus infected or control which were placed in the petri dish. Leaf cores were made from the leaves using a #149 Arch Punch (Osborne and Co., Harrison, NJ). There were three treatments; two viruses and one control. For the soybean looper assays, each treatment consisted of 15 experimental units and was repeated 5 times. Fall armyworm assays consisted of 15 experimental units and were repeated 4 times. Larval feeding was monitored daily and weights were taken after 7 and 14 days. Data analysis was conducted by using PROC Glimmix (SAS Institute Version 9.4).

5.2.6. Oviposition preference assays

Soybean loopers and fall armyworms were sexed during the pupal stage as described by Butt and Cantu (1962). Recently emerged adults (3 males and 3 females) were released into tent-like cages 61 x 61 x 61 cm in dimension (BugDorms, Catalog #1462W, BioQuip Products, Inc., USA) cages, each containing one healthy and one virus infected cowpea test plant in the green-house. Two 30 ml cups containing cotton wadding saturated in 10% honey solution were placed in each cage as supplementary diets for adults (Mascarenhas and Boethel 2000). For soybean looper assays, each experiment consisted of 12 cages (six cages for each virus) and repeated 4 times with a total of 24 replications for each virus. Only 10 cages were used for FAW bioassay and repeated 3 times with a total of 15 replications for each virus. The number of eggs laid on each plant were counted and removed manually each day for a period of 2 weeks after release. Data analysis was conducted by using PROC Glimmix (SAS Institute Version 9.4).

5.3. Results

5.3.1. Feeding bioassays

There were no differences in soybean looper larval weights (both 7-day wt. and 14-day wt.) fed on virus infected and non-infected cowpea ($P > 0.05$) (Table 5.1). However, fall armyworm larvae fed on virus infected cowpea leaf cores showed significant differences in 7-day ($N = 180$, $F = 25.32$, $P < 0.001$) and 14-day larval weights ($N = 180$, $F = 5.80$, $P = 0.0038$). The weights of fall armyworm larvae fed on CMV-infected and ShMV-infected cowpea leaf cores measured in a week were significantly higher as compared to the weights of larvae fed on healthy cowpea leaf cores (Table 5.2). Similarly, comparison of means (Tukey-HSD test, SAS 9.4) showed that fall armyworm gained higher weight in two weeks upon feeding on CMV-infected cowpea as compared to ShMV-infected and sham-inoculated cowpea leaf cores (Table 5.2).

Table 5.1. Summary table showing 7-day wt. and 14-day wt. (g) of soybean looper fed on virus-infected and sham-inoculated cowpea. Values followed by different letters in a column are significantly different by the Tukey-HSD test ($\alpha = 0.05$).

	Soybean looper	
Treatment	7-day wt. (mean ± SE)	14-day wt. (mean ± SE)
Cowpea-CMV	0.0640 ± 0.0057 a	0.1483 ± 0.0200 a
Cowpea-Sham inoculated	0.0596 ± 0.0064 a	0.1469 ± 0.0171 a
Cowpea-ShMV	0.0682 ± 0.0057 a	0.1508 ± 0.0134 a

Table 5.2. Summary table showing 7-day wt. and 14-day wt. (g) of fall armyworm fed on virus-infected and sham-inoculated cowpea. Values followed by different letters in a column are significantly different by the Tukey-HSD test ($\alpha = 0.05$).

	Fall armyworm	
Treatment	7-day wt. (mean ± SE)	14-day wt. (mean ± SE)
Cowpea-CMV	0.1187 ± 0.0043 a	0.241 ± 0.0056 a
Cowpea-Sham inoculated	0.0745 ± 0.0045 c	0.224 ± 0.0058 b
Cowpea-ShMV	0.0992 ± 0.0043 b	0.214 ± 0.0056 b

5.3.2. Oviposition preference assays

Our results showed significant differences among treatments regarding the oviposition preferences by soybean looper adults on cowpea ($N = 96$, $F = 6.55$, $P = 0.0022$). Adult females laid more eggs on healthy cowpea plants as compared to ShMV-infected plants (Table 5.3). Moreover, there was a moderately significant difference in mean number of eggs laid among CMV-infected and healthy plants ($P = 0.0517$). Among all treatments, the mean number of eggs oviposited in healthy plants were higher as compared to virus infected plants (Table 5.3).

The oviposition preference by fall armyworm also followed the same trend. There was a significant difference among the treatments based on oviposition preference by adult fall armyworm females ($N = 60$, $F = 12.42$, $P < 0.001$). The mean number of eggs laid were significantly higher on sham-inoculated plants as compared to plants infected by either of the viruses (Table 5.3).

Table 5.3. Summary table showing total number of eggs laid (mean ± SE) by soybean looper and fall armyworm adults on virus-infected and sham-inoculated cowpea plants. Values followed by different letters in a column are significantly different by the Tukey-HSD test ($\alpha = 0.05$).

	Number of eggs laid (mean ± SE)	
Treatment	Soybean looper	Fall armyworm
Cowpea-CMV	53.9 ± 17.8 ab	41.8 ± 10.9 b
Cowpea-Sham inoculated	96.9 ± 12.6 a	101.9 ± 7.7 a
Cowpea-ShMV	20.1 ± 17.8 b	54.6 ± 10.9 b

5.4. Discussion

Emerging infectious diseases (EIDs) caused by plant pathogens; those that have increased in geographic range, incidence, or pathogenicity or those that have been recently discovered, are on the rise (Anderson et al. 2004). Of EIDs, plant viruses compromise the largest group (47%). Plant viruses can adversely affect agricultural production, being costly to both grower and

consumer by limiting yield and impacting quality. Plant viruses can also alter trophic interactions, enhancing pest herbivore populations. Majority of the researches regarding virus, host plant and herbivores have predominantly focused on interactions between virus and the host plant and between virus and its vectors. Here, we document that plant pathogenic viruses can also impact non-vector herbivores possibly through plant quality changes. Our results indicated that plant viruses didn't affect soybean looper larvae. In a similar study, Lin et al. (2008) reported no effects on feeding and oviposition preferences by *H. armigera* fed on *Tobacco mosaic virus* infected tomato. However, fall armyworm was affected by plant viral pathogens. Fall armyworm larvae benefitted upon feeding on CMV-infected cowpea leaves in our studies. Earlier, Thaler et al. (2010) reported positive effects on growth of *Spodoptera exigua* larvae fed on *Tobacco mosaic virus* (TMV)-infected tomato. CMV infection of *Cucurbita pepo* was reported to cause upregulation of certain plant defense hormones (salicylic acid and ethylene) and downregulation of defense pathways regulated by jasmonic acid (Mauck et al. 2014a). The benefit to caterpillars feeding on a virus infected host, where a salicylate pathway is induced, is presumably due to a weaker ability of the host to induce jasmonate pathway (Thaler et al. 1999, Stout et al. 2006). Similarly, Preston et al. (1999) reported that TMV infected tobacco plants had subdued jasmonic acid induction and decreased resistance to *Manduca sexta*. Our work indicates that as the incidence of plant virus infections rise, this may impact non-vector herbivore performance and may be species specific.

Soybean looper and fall armyworm adults preferred to lay more eggs on the healthy plants as compared to the virus-infected ones. Our current study and the data observed, however, doesn't allow us to decipher how the soybean looper or fall armyworm females were able to detect infected versus non-infected plants and make an oviposition decision. Earlier work has

reported CMV infected plants exhibiting elevated emissions of volatile blends, making them attractive for aphids (Mauck et al. 2010). Our green-house and within cage experimental setting also precluded the understanding of the effects of distance on females while discriminating against infected plants. Nonetheless, this study demonstrates that the effects of virus infection on host plants can have pronounced impacts on plant and non-vector herbivore interactions with consequences for community structure and population dynamics (Mauck et al. 2010). Even though adults preferentially chose to oviposit on healthy plants, fall armyworm larvae seemed to benefit from feeding on virus infected cowpea leaves. These findings are similar to previous published studies by Mauck et al. (2015), who reported that *Anasa tristis* (a specialist non-vector herbivore) adults preferred ovipositing on healthy squash plants although nymphs performed better on CMV-infected squash plants. Based on our results, we propose that plant viruses may be "benefitting" themselves from non-vector herbivores by discouraging adult oviposition in order to ensure their movement and spread by vector herbivores. Further research is required to explicate the mechanisms underlying the observed effects of oviposition preference by females.

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CHAPTER 6: EFFECTS OF *BELL PEPPER ENDORNAVIRUS* ON *MYZUS PERSICAE* HOST PREFERENCE AND POPULATION DYNAMICS

6.1. Introduction

Endornaviruses belong to the genus *Endornavirus* in the family *Endornaviridae* and infect a wide host range including plants, fungi and oomycetes (Hacker et al. 2005, Okada et al. 2013, Li et al. 2014). Endornaviruses are currently classified into two genera, *Alphaendornavirus*, which infects plants, fungi and oomycetes, and *Betaendornavirus*, which infects ascomycete fungi (Adams et al. 2017). Endornaviruses are reported to infect various crops of economic importance, such as, beans, cereals, cucurbits, and peppers (Pfeiffer 1998, Coults 2005, Valverde and Gutierrez 2007). In general, only select cultivars of these crops have been shown to be infected by endornaviruses except for bell pepper (*Capsicum anuum*) and melon (*Cucumis melo*) in the US, which have been reported to be infected almost 100% (Valverde et al. 1990, Okada et al. 2011, Sabanadzovic et al. 2016). Plants infected by endornaviruses are phenotypically normal and do not show any typical viral symptoms (Okada et al. 2011, Song et al. 2013). In addition to plants, endornaviruses have also been described in several species of fungi that include *Alternaria brassicola, Helicobasidium mompa,* and *Tuber aestivum* (Osaki et al. 2006, Stielow et al 2011, Shang et al. 2015) and oomycetes which includes *Phytophthora endornavirus-1* in a *Phytophthora* isolate collected from Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Hacker et al. 2005).

Endornaviruses have a single linear dsRNA genome (9.8-17.6 kbp), are generally transmitted at a high rate only through seeds and are present in a very low copy number (Moriyama et al. 1996, Horiuchi and Fukuhara 2004, Valverde and Gutierrez 2007). Endornaviruses lack both coat protein and movement proteins, and are solely comprised of naked

RNA (Roossinck et al. 2011). Endornaviruses contain only one open reading frame which normally encodes a single polypeptide that is processed by virus-encoded proteinases (Okada et al. 2011). Endornaviruses whose genome have been completely sequenced are reported to possess conserved motifs of RNA-dependent RNA polymerase and viral RNA helicases (Gibbs et al. 2000).

BPEV (*Bell pepper endornavirus*), like other endornaviruses, shows no typical viral disease symptoms on bell peppers (*Capsicum annuum* L.) (Aguilar-Melendez et al. 2009). BPEV produce no apparent symptoms, cannot move from one cell to another but are found at uniform concentrations in every tissue and at every developmental stage of the plant (Okada et al. 2011). Endornaviruses possibly interact with plant hosts in many ways: i) parasitic, and use host resources for their replication, ii) mutualistic, a possibility looking at the high rate of vertical transmission from parent to progeny suggesting a selection for the endornavirus infection, or iii) commensalistic, benefitting the virus while no effects on their hosts (Herschlag 2017).

Although reported infecting many economically important crops, the effects of endornaviruses on their hosts have not been fully studied primarily due to the lack of a practical inoculation method (Khankhum and Valverde 2018), resulting in very few studies conducted to assess the effects of endornaviruses on their hosts. A positive effect on seed germination, longer sized pods, and higher seed weight were reported on endornavirus-infected common bean (*Phaseolus vulgaris*) as compared to endornavirus-free common bean plants (Khankhum and Valverde 2018). On the contrary, a negative effect of BPEV on bell pepper was also reported (Escalante 2017). Using two near-isogenic lines (NILs) of the bell pepper cv. Marengo, Escalante (2017) conducted a comparative study on plant physiology and phenotypic characteristics and reported that fruits obtained from BPEV-free pepper plants weighed

significantly higher than the fruit weight obtained from BPEV-infected line. Similarly, a higher percentage of seed germination and root length was measured in BPEV-free lines. However, no statistically significant differences were observed on stem thickness, height, fruit size, and dry matter percentage of the plants among BPEV-infected and BPEV-free lines (Escalante 2017).

Moreover, Escalante (2017) also investigated the interaction of BPEV with *Pepper mild mottle virus* (PMMoV) and found that BPEV-infected lines had less severe symptoms and lower virus titer and viral RNA accumulation, suggesting an antagonistic relationship between BPEV and PMMoV. Earlier, Valverde and Fontenot (1991) had reported the presence of BPEV in all tested bell pepper cultivars. It appears that plant breeders while developing bell pepper cultivars, inadvertently selected and introduced virus infected genotypes in bell pepper growing regions, completely unaware of the presence of endornaviruses in the germplasm. Because BPEV is transmitted vertically and is at constant concentrations in almost all commercial bell pepper cultivars, it is logical to presume that BPEV might also be providing a benefit. One of the probable beneficial effects could include resistance or tolerance to biotic and abiotic factors. Therefore, endornaviruses may have evolved a symbiotic relationship with their hosts to tolerate stresses. With this in mind, we set out experiments to determine host suitability and population dynamics of green peach aphid on BPEV-infected and BPEV-free pepper test plants.

6.2. Materials and Methods

6.2.1. Host plants, Virus source and maintenance

Two near isogenic lines (NILs) of bell pepper cv. Marengo, one BPEV-infected and another BPEV-free, were developed in the Plant Virology Laboratory (Department of Plant Pathology and Crop Physiology under the direction of R. Valverde) at LSU as described by Guardado (2017). The plants were planted in steam sterilized soil mixture consisting of soil, sand, and

Miracle-Gro potting mix (Scotts Miracle-Gro, Marysville, OH) in a proportion of 2:1:1. The plants were grown in the greenhouse at an average temperature of 28 ℃. For the detection of virus, viral double stranded RNA (dsRNA) was extracted as described by Khankhum et al. (2017), analyzed in agarose gel electrophoresis, and used in reverse-transcription polymerase chain reactions (RT-PCR) as reported by Okada et al. (2011). The plants, after confirmatory tests, were obtained from the Plant Virology Laboratory and maintained in the greenhouse at 22-28 ℃ and a varying RH. The plants were kept separately inside tent-like cages 61 x 61 x 61 cm in dimension (BugDorms, Catalog No. 1462W, BioQuip Products, Inc., USA) in order to ensure no cross contamination.

6.2.2. Aphid colony

Green peach aphids used in the experiment came from a colony established from a single apterous *M. persicae* collected from eggplant (*Solanum melongena* L.) in 2009. The colony was maintained in screened cages (30 x 30 x 30 cm, assembled using Plexiglass plastic sheet and nylon mesh fabric) and reared on mustard (*Brassica juncea* L.) cv. Tendergreen (W. Atlee Burpee and Co., Warminster, PA), under laboratory conditions at room temperature (20-22 °C) and a 14:10 (L: D) photoperiod. Mustard plants were planted in plastic pots 10 cm in diameter (Dillen Products, Middlefield, OH) containing sterile potting mix (Miracle-Gro Organic Choice Garden Soil, Marysville, OH) and 5g Osmocote (14:14:14) (The Scotts Company, Marysville, OH) and grown in growth chambers (Percival Scientific, Perry, IA) maintained at 25 ± 2 °C, 50 ± 5% RH and a photoperiod of 14:10 (L: D). A cohort of 10 to 15 aphids was placed on fresh plants every 2 to 3 weeks to establish and maintain a new colony.

6.2.3. Choice bioassay

Host plant selection preference of adult apterous non-viruliferous *M. persicae* was examined using a dual-choice bioassay arena. The plants used in the choice bioassay were near isolines of each other, differing only in infection status. The leaves were carefully excised along with the petiole from the plants in the greenhouse and brought to the laboratory for the experiment. The choice bioassay arena consisted of 3 circular petri dishes, one at the center (central) and two at the edges (peripheral) (Fig 6.1). Each peripheral petri dish consisted of two holes (approximately 0.5 cm in diameter), one to connect with the central petri dish, and another to insert the excised treatment leaf into the arena. The central petri dish consisted of two holes to insert tubes in order to connect with two petri dishes in the opposite sides. The petiole of the excised treatment leaf was inserted in a glass tube containing water and sealed with parafilm (Parafilm, Pechiney plastic packaging, Menasha, WI) in order to avoid wilting of the leaf due to desiccation during the experimental duration. 50 adult apterous aphids were removed from the colony with the help of a fine camel's hair brush and placed in 2-ml micro-centrifuge tube. After a starvation period of 2 h, aphids were carefully released into the central petri dish and allowed to choose between BPEV-infected or BPEV-free bell pepper leaves placed at two different peripheral petri dishes. Aphid observations were made at 0.5 h, 1 h, 2 h, 6 h, and 24 h after release into the arena and the number of aphids were counted during each observation period. The experiment was conducted under laboratory conditions at room temperature (20-22 °C) and a 14:10 (L: D) photoperiod. The positions of leaves were varied during each replication to avoid continuous placing of one treatment on one side of the arena. The experiment was repeated 20 times. The proportion of aphids responding to either treatments were compared using a generalized linear mixed model assuming a binomial distribution with a logit link function in

SAS (Proc Glimmix, SAS Institute version 9.4, Cary, NC). The time variable examined observations made at different intervals after release using a repeated measures design. Treatment means were separated by the Tukey-HSD test ($\alpha = 0.05$). Aphids not located on either plant leaf in an arena were considered non-responsive and excluded from the analysis.

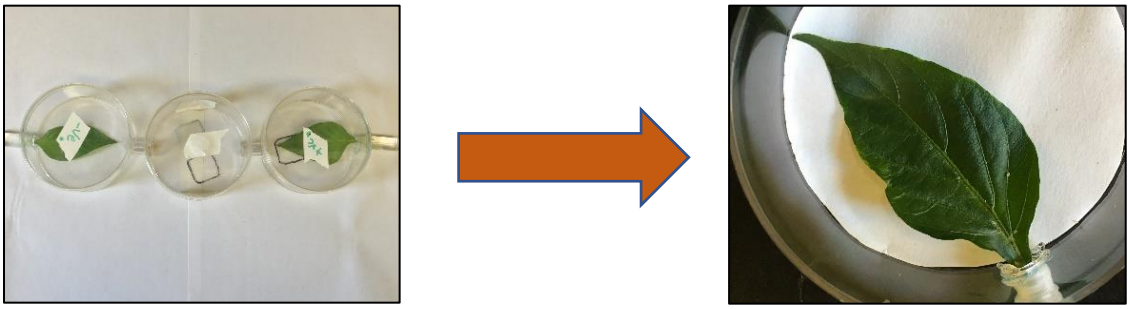

Fig. 6.1. Showing the experimental setup of the dual choice bioassay (left) and a close-up of the arena (right).

6.2.4. Life table studies

The life table studies of *M. persicae* on two different hosts (NILs), BPEV-infected and BPEV-free on bell pepper cv. Marengo was conducted and aphid life history traits were compared on the host plants using excised leaves. Plant leaves were carefully excised from the plants in the green house and brought to the laboratory. Circular leaf cores (approximately 11.3 cm^2) were cut from the leaves using a #149 Arch Punch (Osborne and Co. Harrison, NJ). Leaf cores were placed in 30 ml Solo cups (Dart Container Corporation, Mason, MI). The cups were filled previously with 12-15 ml agarose solution (0.1% w/v) (RM3001-500G Agar Powder Extra Pure, HiMedia, Einhausen, Germany). Leaf cores were placed with abaxial surface upward on top of the agarose solution. Agarose solution helped to avoid dehydration of the leaves and prevented aphids from escaping the leaf surface. Adult apterous aphids were removed from the colony and placed in individual cups with the help of a fine camel's hair brush and allowed to larviposit for 24 h. After 24 h, adult aphid along with all nymphs but one were removed so that only one 1st

instar nymph remains in each cup. All single 1st instar nymphs consisted the cohort for that treatment for the experimental duration. There were 25 nymphs per cohort and the experiment was replicated 4 times for both the treatments. The cohort was examined every 24 hours for deaths, change of instars, and nymphs produced per day until death for all individuals. Life table parameters were calculated for each host plant as reported by Birch (1948). Age-specific survival *(l_x)*, the proportion of live females on a given day, and fecundity *(m_x)*, the mean number of births by female on that day were calculated for each treatment host. Net reproductive rate, R_0, defined as the product of age-specific survival and age-specific fecundity was calculated using the formula $R_0 = \sum l_x m_x$. The intrinsic rate of increase, r_m, was calculated as, $\sum e^{-r_m} l_x m_x = 1$. Finite rate of increase was calculated as $\lambda = e^{r_m}$, Mean generation time was calculated as $T_G = \ln R_0/r_m$, and doubling time was calculated as, DT = ln (2)/r_m. Jackknifing procedure was used to estimate r_m standard error. Jackknifing is based on calculating the pseudo-values after recombining the original data and estimating the mean value and standard error of r_m from the frequency distribution of pseudo-values (Meyers et al. 1986). Pseudo-values of r_m were used to estimate the values for net reproductive rate, finite rate of increase, mean generation time and population doubling time and these variables were analyzed by one-way analysis of variance in SAS (Proc Glimmix, SAS Institute version 9.4, Cary, NC). Treatment means were separated by the Tukey-HSD test ($\alpha = 0.05$). The biological variables (days to reproductive adult, reproductive period, longevity, progeny per female, and progeny per female per day) were analyzed by one-way analysis of variance in SAS (Proc Glimmix, SAS Institute version 9.4, Cary, NC) and treatment means for each variable were compared using Tukey-HSD at 0.05% significance level. Age-specific survivorship and fecundity graphs were plotted using Excel (Microsoft Excel Ver. 3, 2013).

6.3. Results

6.3.1. Choice bioassay

Aphid preference behavior experiments through choice bioassay arena showed that apterous *M. persicae* tended to prefer BPEV-free bell pepper leaves than BPEV-infected leaves (Table 6.1). The percentage of aphids observed on BPEV-free leaves were significantly higher than those observed on BPEV-infected leaves throughout the experimental period. This suggests that aphids preferred to feed on healthy leaves as compared to BPEV-infected ones.

Table 6.1. Analysis of variance (ANOVA) test results showing the differences in mean proportion of aphids (Means ± S.E.) among the aphids responding to either treatments observed at different observation points throughout the experiment. Values followed by different letters in a row are significantly different by Tukey-HSD test ($\alpha = 0.05$).

Observation point	Proportion of aphids observed in different hosts	
	BPEV-free	BPEV-infected
0.5 h	0.92 ± 0.03 a	0.08 ± 0.03 b
1.0 h	0.74 ± 0.04 a	0.26 ± 0.04 b
2.0 h	0.62 ± 0.05 a	0.38 ± 0.05 b
6.0 h	0.60 ± 0.04 a	0.40 ± 0.04 b
24.0 h	0.59 ± 0.05 a	0.41 ± 0.05 b

6.3.2. Life table analysis

Life table analysis through feeding bioassays on BPEV-infected and BPEV-free bell pepper showed statistically significant differences of the biological variables (Table 6.2). Aphids fed on BPEV-free bell pepper leaf cores had a greater longevity (average life span from day one until death) than those fed on BPEV-infected leaf cores ($F = 3.966$, $P = 0.048$). Similarly, differences were statistically significant for two reproductive variables, progeny per female ($F = 6.144$, $P = 0.014$) and progeny per female per day ($F = 9.087$, $P = 0.003$), where aphids performed better upon feeding on BPEV-free leaf tissue as compared to BPEV-infected ones. However, no

significant differences were observed on biological variables days to reproductive adult and reproductive period (the duration in which female remained reproductively active) ($P > 0.05$).

Statistically significant differences were observed in intrinsic rate of increase (r_m), which was higher in BPEV-free treatment as compared to BPEV-infected one. All other population parameters were not statistically different among treatments (Table 6.2).

Table 6.2. Life-table parameters (mean ± SE) of *M. persicae* on BPEV-infected and BPEV-free bell pepper cv. Marengo. Values followed by different letters in a row are significantly different by Tukey-HSD test ($\alpha = 0.05$).

	Host plant	
Parameters	BPEV-free	BPEV-infected
Biological parameters		
Days to reproductive adult	7.6 ± 0.1 a	7.5 ± 0.1a
Reproductive period	5.0 ± 0.3 a	4.6 ± 0.3 a
Longevity	12.2± 0.4 a	11.1 ± 0.4 b
Progeny per female	19.4 ± 1.2 a	15.1 ± 1.2 b
Progeny per female per day (m_x)	3.7 ± 0. a	3.1 ± 0.1 b
Population parameters		
Intrinsic rate of increase (r_m)	0.343 ± 0.042 a	0.273 ± 0.019 b
Net reproductive rate (R_0)	13.7 ± 3.6 a	9.2 ± 2.3 a
Finite rate of increase (λ)	1.4 ± 0.1 a	1.3 ± 0.1 a
Doubling time (D_T)	2.0 ± 0.3 a	2.5 ± 0.3 a
Mean generation time (G_T)	7.6 ± 0.4 a	8.1 ± 0.3 a

Age specific fertility rate (m_x) curve was higher for BPEV-free bell pepper as compared to BPEV-infected one (Fig. 6.2). Similarly, age-specific survival rate (l_x) curve was also higher for BPEV-free bell pepper as compared to BPEV-infected bell pepper (Fig. 6.3).

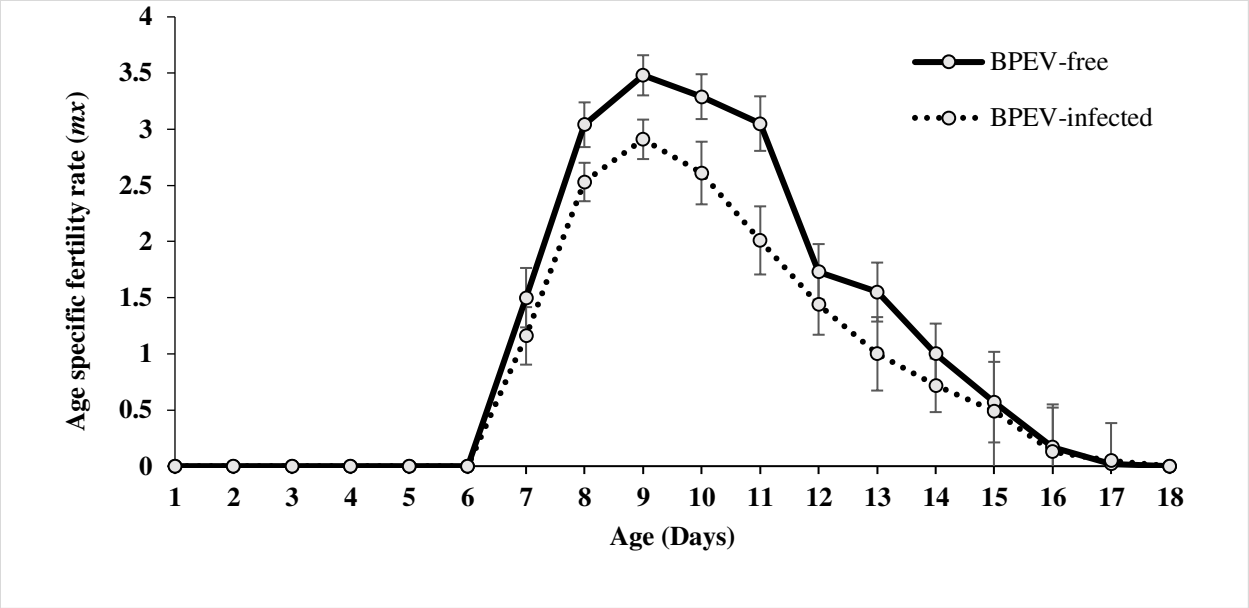

Fig. 6.2. Age-specific fertility rate of *M. sacchari* on BPEV-infected and BPEV-free bell pepper cv. Marengo.

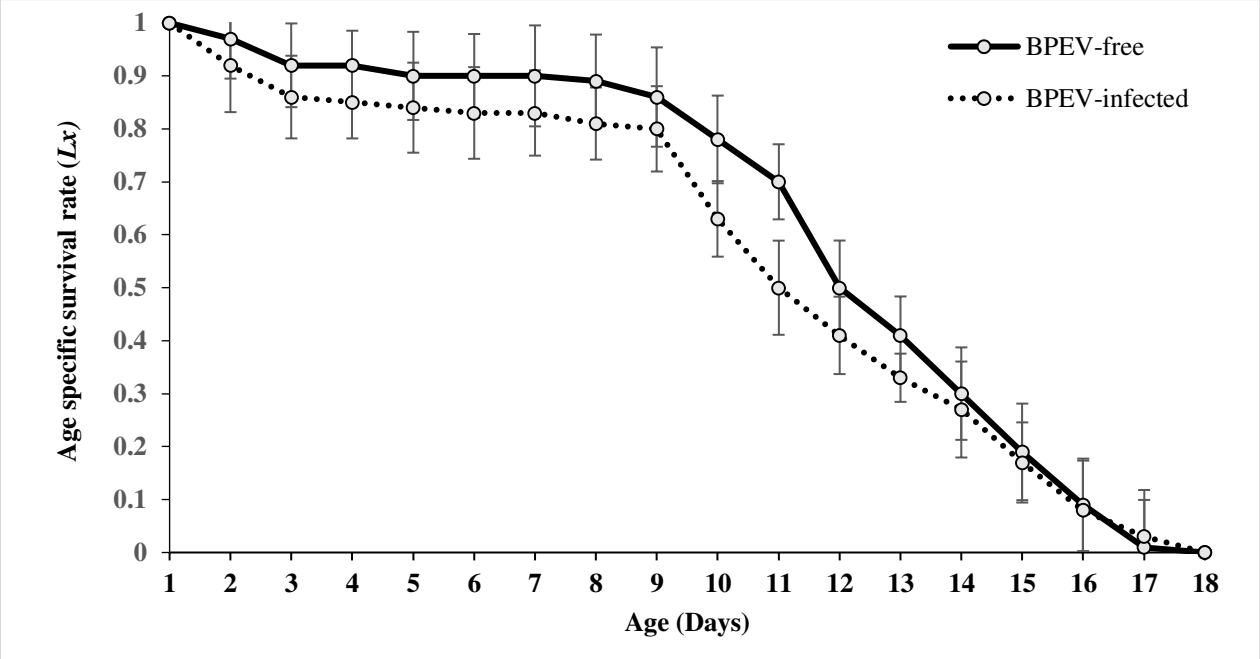

Fig. 6.3. Age-specific survival rate of *M. sacchari* on BPEV-infected and BPEV-free bell pepper cv. Marengo.

6.4. Discussion

Since the advent of plant viruses and their basic understanding as pathogens, a majority of the studies have concentrated on the negative impacts of viruses on plants. However, many viruses are found to be beneficial to their hosts either by enhancing the host ability to counteract abiotic stresses, or in some cases, altering the plants ability to deal with biotic stresses (Roossinck, 2015). The effects of plant viruses, in some cases, can be conditional as stated by Bao and Roossinck (2013), where virus, under normal conditions, may be a pathogen but benefit the hosts under stress. Plant viruses induce specific changes in plant volatile emission and alter the physiology and the morphology of the plant (Bosque-Perez and Eigenbrode 2011, Mauck et al. 2012). BPEV, a persistent virus in bell pepper has been maintained at a constant concentration and transmitted vertically by the host plants in almost all commercial bell peppers varieties (Valverde et al. 1990, Okada et al. 2011). However, the impacts of BPEV on bell pepper and insect pests that attack bell pepper have rarely been studied. Escalante (2017) reported a negative effect of BPEV, in which fruits from BPEV-free bell pepper weighed higher than fruits from BPEV-infected bell pepper plants. In contrast to this result, Escalante (2017) reported that presence of BPEV had an antagonistic relationship on *Pepper mild mottle virus* (PMMoV), a disease-causing virus on pepper, where BPEV-infected lines had less severe symptoms due to PMMoV, lower virus titer and viral RNA accumulation.

Here, we report the first instance of effects of BPEV on preference behavior, growth and reproduction of an important insect pest on bell pepper under laboratory conditions. In our study, we examined the potential interaction between BPEV and *M. persicae*, a common pest of pepper (Blackman and Eastop 1984, Frantz et al. 2004). During host preference bioassays, we found that higher number of aphids were observed initially and throughout the observation period on BPEV-free leaf arena. This could indicate that BPEV infection might have benefitted the host by

making them unattractive to the herbivores and could be reducing virus inoculation of non-persistent viruses. Our experiment was not designed for aphids to move freely between treatments and make a feeding decision. So, there is no evidence to entail that aphid's behavior was altered upon feeding on BPEV-infected leaf tissue. However, a rapid efflux of aphids from either treatment arena was not observed in order to suggest an alteration in behavior after feeding on a particular host. Also, it is not surprising for BPEV to have no effects on *M. persicae* behavior upon feeding since it is a non-vector of BPEV. We also found that BPEV negatively affected longevity and fecundity of aphids. Population parameters like intrinsic rate of increase, and mean generation time were negatively affected by BPEV. Similarly, age specific survival rate and fertility rate were higher on aphids fed on BPEV-free leaf tissues. The findings of this study contribute an important first stride towards understanding the intricate interactions that occur between BPEV, bell pepper and *M. persicae*. The mechanism for decreased population growth rate is not clear but life table analyses suggest that decreased survival rate on infected BPEV might lead to decreased aphid performance in the field. Lower population growth rate upon feeding on BPEV-infected plants can help to check the rapid growth of pest population and allow time for other control measures to come into effect for successful management of the pest. Moreover, since the negative effects of BPEV on bell pepper have not been completely understood and the interactions seem rather beneficial to the host, this opens a new avenue for the integration of BPEV-infected pepper plants in the crop ecosystem. Other insect pests that attack bell pepper besides *M. persicae* are beet armyworm (*Spodoptera exigua*), flea beetles, leafminers, leafrollers, pepper weevil (*Anthonomus eugenii*), thrips, and tomato fruitworm (*Helicoverpa zea*). Similarly, fungal diseases like anthracnose, damping-off, mildew, blight, cercospora leaf spot, bacterial diseases like bacterial spot, bacterial wilt, and viral disease like

(*Cucumber mosaic virus*, *Potato virus Y*, *Tomato spotted wilt virus*) also attack bell peppers. Therefore, understanding the interactions between the aforementioned biotic factors and BPEV-infected bell pepper is very important to understand in integrated pest management programs.

6.5. References

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CHAPTER 7: SUMMARY AND CONCLUSION

Plant virus-vector-host are complex and the interactions between key components in the pathosystem are very important in order to devise sound management strategies for the control of plant virus disease epidemiology in the landscape. Although the interactions between plant viruses and their impacts on the behavior and performance of their vector herbivores are studied in many pathosystems, there is a dearth of information regarding the impacts of plant viruses on non-vector herbivores. This is crucial because the majority of insect herbivores are non-vectors and the impacts plant viruses might have on non-vector herbivores could lead to the reassessment of prevalent pest management options. Moreover, feeding on an infected plant, even if an insect might not transmit a specific pathogen, could have major impacts on its behavior with significant implications for its ecology.

In order to shed some insights on the interactions between plant viruses, their hosts, potential vectors, and non-vector herbivores, three unique pathosystems were chosen for our studies. At first, we conducted experiments in *Sorghum mosaic virus* (SrMV)-sorghum (*Sorghum bicolor* L.) pathosystem. Transmission studies for SrMV in sorghum were conducted for the first time on sugarcane aphid (*Melanaphis sacchari* Zehntner), an important invasive pest in U.S. sorghum, and green peach aphid (*Myzus persicae* Sulzer), a model vector for non-persistently transmitted viruses. We also conducted electrical penetration graph (EPG) studies to understand the feeding behavior and choice bioassay experiments to understand the preference behavior of *M. persicae* and *M. sacchari* on SrMV-infected and non-infected sorghum. Life history and population dynamics of *M. sacchari* on SrMV-infected and non-infected sorghum were also studied.

We found that transmission of SrMV differs among two aphid species; *M. sacchari* and *M. persicae*. *M. sacchari* failed to transmit SrMV both singly and in groups under laboratory conditions. Based on our results, we state that *M. sacchari* is a non-vector of SrMV in sorghum. Therefore, it is safe to mention that sorghum growers do not need to be concerned regarding the indirect damage that sugarcane aphid can inflict through transmission of SrMV. *M. persicae*, however, successfully transmitted SrMV in sorghum in our studies at different efficiencies (4.2 to 8.2 %), depending upon whether they were allowed to transmit singly or in groups. The non-persistent transmission like that of SrMV is best accomplished by non-host aphid species like *M. persicae*, which are known to be capable of causing severe mosaic epidemics in sugarcane. Moreover, the incidence and increase of virus disease also depends on the number of migrating aphid vectors passing through the landscape. Therefore, the ability of *M. persicae* to cause SrMV disease epidemiology on sorghum fields should not be underestimated. Studies of feeding behavior by EPG suggested that by virtue of producing higher numbers of probes during feeding, a longer potential drop duration with longer subphases II-1 and II-3, higher number of archlets during subphase II-3, and quicker to produce first potential drops than *M. sacchari*, *M. persicae* possesses an ability to successfully transmit SrMV from sorghum to sorghum.

Our second study system consisted of *Cucumber mosaic virus* (CMV) or *Sunn-hemp mosaic virus* (ShMV) infected cowpea (*Vigna Unguiculata* (L.) Walp) and its interaction with two non-vector herbivores, soybean looper, *Chrysodeixis includens* (Walker) and fall armyworm, *Spodoptera frugiperda* (Smith). We conducted greenhouse and laboratory experiments to determine the effects of *Cucumber mosaic virus* or *Sunn-hemp mosaic virus* infected cowpea on soybean looper and fall armyworm larval growth and adult oviposition preference. We found that both viruses didn't affect soybean looper larval growth. However, fall armyworm larvae

benefitted upon feeding on CMV-infected cowpea leaves in our studies. Here, we document that plant pathogenic viruses can also impact non-vector herbivores possibly through plant quality changes. Though our study was not designed to study the possible cause for this effect, the benefit to caterpillars feeding on a virus infected host, where a salicylate pathway is induced, might presumably be due to a weaker ability of the host to induce jasmonate pathway. In the oviposition preference study, we observed that soybean looper and fall armyworm adults preferred to lay more eggs on the healthy plants as compared to the virus-infected ones. This demonstrates that the effects of virus infection on host plants can have significant impacts on plant and non-vector herbivore interactions with implications for community structure and dynamics. Based on our results, we propose that plant viruses may be "benefitting" themselves from non-vector herbivores by discouraging adult oviposition and subsequent larval feeding on the infected hosts in order to ensure their movement and spread by vector herbivores.

Lastly, the third pathosystem we studied consisted of a persistent virus, *Bell pepper endornavirus* (BPEV) in bell pepper (*Capsicum annuum* L.). BPEV is a persistent virus with no known vectors, is transmitted only vertically, and is present at constant concentrations in every tissue in all tested bell pepper cultivars. BPEV produces no symptoms and no negative effects have been documented on the infected hosts. There seems to have evolved a symbiotic relationship between virus and the host suggesting that viruses might be providing a benefit in terms of tolerance or resistance to biotic and abiotic agents. Thus, we conducted lab experiments to understand if BPEV-infection provides any benefit to bell pepper hosts against a common pest, *M. persicae*. We designed experiments to determine preference behavior, host suitability and population dynamics of *M. persicae* on BPEV-infected and non-infected pepper plants. Through host preference bioassays, we found that *M. persicae* preferred virus-free leaves as

compared to virus infected ones. This could indicate that BPEV infection might have benefitted the host by making them unattractive to the vector herbivores and could be reducing virus inoculation of non-persistent viruses. Moreover, we also found that BPEV negatively affected longevity and fecundity of aphids. The findings of this study provide an important first step towards understanding the complex interaction that occur between BPEV, bell pepper and *M. persicae*.

In conclusion, in our study in SrMV-sugarcane aphid-sorghum pathosystem, we observed that plant virus negatively affected the population dynamics of a piercing sucking herbivore. A similar result was obtained in the BPEV-green peach aphid- bell pepper pathosystem. In addition, oviposition of fall armyworm and soybean looper, two economically important chewing herbivores in CMV-cowpea and ShMV-cowpea pathosystems were also negatively affected by virus infected cowpea plants. In contrast to these results, the larval growth of fall armyworm was positively affected upon feeding on CMV infected cowpea leaves. Thus, it is difficult to draw a broader conclusion regarding the impacts of plant viruses on non-vector herbivores, which might be virus-insect specific. More research is needed in order to fully understand the interaction among various components in a diverse plant virus-host-insect agroecosystem.

VITA

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