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Dynamics of the sweetpotato potyvirus aphid pathosystem in Louisiana

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**DYNAMICS OF THE SWEETPOTATO POTYVIRUS APHID
PATHOSYSTEM IN LOUISIANA**

A Dissertation

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

in

The Department of Plant Pathology and Crop Physiology

by

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December 2012

DEDICATION

This dissertation is dedicated to my son Moses Kipseete and my husband Erastus Chepkwony for their love and support, to my beloved grandparents Dina Nasipwondi and Jeremiah Wosula who taught me the values of life and the importance of education. I also dedicate this work to my mum, my sister Tatiana, my brother Phillippe, my aunt Charity and to all my extended family members for their prayers, love and moral support.

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ABSTRACT

Sweetpotato potyviruses [*Sweet potato feathery mottle virus* (SPFMV), *Sweet potato virus G* (SPVG) and *Sweet potato virus 2* (SPV2)] commonly infect sweetpotato and weedy morning glories in the USA. These viruses are transmitted in a non-persistent manner by various aphid species and cause up to 15% yield loss. Sweetpotato is vegetatively propagated, and in the USA growers are supplied with virus tested propagation material to minimize impact of viruses. However the rapid re-infection of these materials with viruses warranted further studies to determine factors that influence the epidemiology of these viruses. The objectives of this study were: (i) to determine if differences in acquisition hosts, aphid species and infection status influenced transmission of SPFMV; (ii) to determine how aphid abundance, aphid species diversity and virus titers relate to the spread of potyviruses in Louisiana sweetpotato fields; (iii) to determine the effects of virus infection on the population dynamics of aphids on sweetpotato and morning glories; and (iv) to determine the effects of virus infection on stylet penetration behaviors of aphids. SPFMV was transmitted at a greater rate from morning glories which also had greater virus titers compared with sweetpotato and from mixed infection sources than from singly infected sources, and *Aphis gossypii* was the most efficient vector. Aphids were captured in fields during the entire crop cycle, and *A. gossypii* and *Rhopalosiphum padi*, were the most abundant species occurring throughout the growing season. Virus infection of sentinel plants occurred mainly during the months of June to August when virus titers were high in sweetpotato plants. SPFMV was more commonly detected than SPVG or SPV2 in sentinel plants. *Myzus persicae* had a significantly greater reproduction on sweetpotato cvs. Beauregard and Evangeline with

mixed virus infection compared with non-infected plants. Stylet penetration behaviors were variable depending on host and virus infection status. Differences in virus transmission rates depending on host plant, aphid species, virus species and virus titers, and pattern of spread in sweetpotato fields suggest the dissemination of sweetpotato potyviruses is influenced by the source of inoculum, the quantity of inoculum, virus species and aphid species vectors.

CHAPTER 1: INTRODUCTION

Sweetpotato [*Ipomoea batatas* (L.) Lam.] is the seventh most important food crop in the world with an annual production of approximately 130 million tons, ranking third among root and tuber crops worldwide (FAO, 2005). It is increasing in demand in the USA given its perception as a nutritious food with more processed products becoming available (Clark et al., 2010). Sweetpotato is an important carbohydrate source, especially in Africa, where it provides household food security, because it persists well in the soil as a famine reserve crop and performs well in marginal soils (Byamukama et al., 2004; Clark et al., 2010).

Sweetpotato is vegetatively propagated and is prone to accumulate viruses which cause cultivar yield decline and reduce storage root quality (Clark et al., 2002; Clark and Hoy, 2006). The most common sweetpotato viruses in the USA are members of the family *Potyviridae* and the genus *Potyvirus*: *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato virus G* (SPVG) and *Sweet potato virus 2* (SPV2, synonym = *Ipomoea vein mosaic virus*) (Clark et al., 2012; Clark and Moyer, 1988; Souto et al., 2003). Sweetpotato potyviruses are restricted primarily to the family *Convolvulaceae*, transmitted by aphids, and occur commonly as mixed infections in the field (Byamukama et al., 2004; Moyer and Salazar, 1989; Stubbs and McLean, 1958).

SPFMV is the most common sweetpotato virus and occurs in all sweetpotato growing areas including the USA (Clark and Moyer, 1988). Four strains were originally differentiated: russet crack (RC), common (C), ordinary (O) and East African (EA) (Kennedy and Moyer, 1982; Kreuze et al., 2000). The RC and C strains are widely distributed, while EA seems to be most common in Africa, though it has also been

reported in Peru, Spain and Easter Island (Gutiérrez et al., 2003; Kreuze et al., 2000; Rännäli et al., 2009; Valverde et al., 2004). However, the C strain was recently separated into a distinct species, *Sweet potato virus C* (SPVC), due to its sequence divergence from the other three strains (Untiveros et al., 2010).

Mixed virus infections can positively or negatively impact transmission rates and alter patterns of virus spread (Rochow, 1972). Mixed infections could be better sources of inoculum for some viruses, while for others transmission rates are greater from single infections (Rochow, 1972). In Louisiana, after one generation in the field, plants are commonly infected with SPFMV, but by the fourth generation, plants usually test positive for SPFMV (100%), SPVG (50 to 70%) and SPV2 (25 to 30%) (Clark et al., 2010). Since SPVC was not recognized as a distinct species until recently, data are not available for its occurrence.

Experimental host range studies suggest that other wild virus hosts are potential sources of inoculum (Clark and Moyer, 1988) mainly morning glory plants in the genus *Ipomoea*, family *Convolvulaceae* (Loebenstein et al., 2009; Tugume et al., 2008). In Louisiana, the morning glories *Ipomoea cordatotriloba* Dennst. (synonym *I. trichocarpa* Ell.) and *I. hederacea* Jacq. are common in and around sweetpotato fields, and *I. hederifolia* L., *I. lacunosa* L., and *I. wrightii* (Wall.) are also known wild hosts of SPFMV (Clark et al., 1986).

Sweetpotato potyviruses are transmitted by several aphid species (Stubbs and McLean, 1958) in a non-persistent manner (Kennedy et al., 1962; Kennedy and Moyer, 1982). Aphid vectors acquire and transmit potyviruses to susceptible plants without necessarily colonizing the infected plants due to their sap sampling/probing feeding habit

(Powell et al., 2006). These aphids acquire the virus in as short as 3 to 35 seconds (McLean, 1959), and retain the virus for a short period, normally less than two hours (Ng and Falk, 2006). Efficient vectors of SPFMV are the aphid species *Myzus persicae* (Sulzer) and *Aphis gossypii* Glover (Byamukama et al., 2004; Stubbs and McLean, 1958). *Myzus persicae* occasionally colonizes sweetpotato in Louisiana, while *A. gossypii* has been found on *I. hederacea* (Clark, C. A. personal communication).

Aphid flight activity and species diversity have been monitored in other crop systems with the aim of understanding virus spread, and timing of control strategies such as applications of insecticides, crop oils or defoliant (Radcliffe and Ragsdale, 2002). In sweetpotato, the only recent study of aphid activity has been one related to the spread of sweet potato virus disease (SPVD) which is caused by synergism between the aphid-transmitted SPFMV and whitefly-transmitted *Sweet potato chlorotic stunt virus* (SPCSV) (Clark et al., 2012; Valverde et al., 2007). Byamukama et al. (2004) found that aphids were present throughout the study period in Uganda. The authors did not report on aphid species diversity, but emphasized more on the relationship between whiteflies and spread of SPCSV rather than aphid spread of SPFMV. In Louisiana during the 1950s, Kantack et al. (1960) trapped aphids using tangle foot traps in sweetpotato fields in south Louisiana. The authors captured several aphid species, with the majority being *A. gossypii*, *M. persicae* and *Macrosiphum euphorbiae* (Thomas).

Plant pathogen-vector system interactions can have both direct and indirect effects. The possible components through which the systems could interact include: dependence of the virus on the arthropod vector for transmission, pathogen effect due to its presence and replication in the vector, pathogen and vector competition for limiting

resources, and pathogen and vector potential to induce host defense mechanisms hence affecting each other indirectly through the response of the plant (Belliere et al., 2005). Vector biology and ecology is, in most cases, neglected when dealing with virus epidemiology (Radcliffe and Ragsdale, 2002). Aphids reported to colonize sweetpotato include *Aulacorthum solani* (Kalt.), *A. gossypii*, *Aphis nasturtii* (Kalt.), *Aphis spiraecola* (Patch), *M. euphorbiae* and *M. persicae* (Blackman and Eastop, 2006), but their reproductive behavior on this crop is unknown. Since most sweetpotato plants in Louisiana growers' fields are infected with potyviruses (Clark et al., 2010), there is a high probability that colonizing aphids will encounter virus-infected plants.

Probing and feeding behavior by aphids and other piercing and sucking insects can be monitored by means of electronic devices (Ferreles and Moreno, 2009). McLean and Kinsey (1964) developed the first equipment, but these techniques have been improved to give more precise and relevant information on the insect activities and position of the stylet inside the plant (Backus and Bennett, 1992; Tjallingii, 1988). Electronic devices based on DC-amplifiers commonly referred to as electrical penetration graph (EPG) can distinguish between the intercellular and intracellular environment, which makes it possible to know when plant cells are punctured by insect stylets (Tjallingii, 1985). Electrical penetration graph techniques (Tjallingii, 1988) have been used widely to study host plant resistance to aphids (Davis and Radcliffe, 2008; Diaz-Montano et al., 2007; Montllor and Tjallingii, 1989; van Helden and Tjallingii, 2000) as well as effect of several behavior modifying compounds (Nisbet et al., 1993; Powell, 1992). Electrical penetration graph techniques have also been used to study non-persistent virus transmission (Collar et al., 1997).

1.1 Justification

Sweetpotato yield and quality in the USA are negatively affected by a complex of potyviruses that include *Sweet potato feathery mottle virus*, *Sweet potato virus G* and *Sweet potato virus 2*. Virus-tested plants are frequently re-infected with viruses by the end of the growing season, especially with SPFMV. This rapid re-infection compromises efforts made by the seed certification program to supply growers with uninfected material. Thus a need exists to establish factors that are involved in the spread of these potyviruses.

1.2 Objectives

The objectives of this study were:

- To determine if differences in acquisition hosts (sweetpotato and morning glory), aphid species (*Myzus persicae* and *Aphis gossypii*) and infection status (single vs. mixed infection) influenced transmission of SPFMV.
- To determine how aphid abundance, aphid species diversity and virus titers relate to the spread of potyviruses in Louisiana sweetpotato fields.
- To determine the effects of virus infection on the population dynamics of *M. persicae* on sweetpotato (cvs. Beauregard and Evangeline) and morning glories (*I. cordatotriloba* and *I. hederacea*).
- To determine the effects of virus infection on stylet penetration behaviors of *M. persicae* associated with non-persistent virus transmission and host acceptance on sweetpotato (cvs. Beauregard and Evangeline) and morning glories (*I. cordatotriloba* and *I. hederacea*).

CHAPTER 2: LITERATURE REVIEW

2.1 Sweetpotato

2.1.1 Origin and taxonomy

The sweetpotato [*Ipomoea batatas* (L.) Lam.] was domesticated about 5000 years ago in tropical America (Austin, 1988). According to Austin (1988) sweetpotato may have originated in the region between the Yucatan Peninsula of Mexico and the Orinoco River in Venezuela. Recent studies on diversity assessment using molecular markers found the highest diversity in Central America and supported the hypothesis that this region is the primary center of diversity and most likely the center of origin of sweetpotato (Huang and Sun, 2000; Zhang et al., 2000). The crop was introduced to other world regions including Africa, India, Southeast Asia and China during the 16th century (O'Brien, 1972; Yen, 1982). The sweetpotato became widely grown in North America by end of 18th century due to dissemination by explorers from Mexico and West Indies (Edmond, 1971).

Sweetpotato is a dicotyledonous plant which belongs to the family *Convolvulaceae*. In the family there are approximately 50 genera and more than 1200 species. The plants of this family mostly have the following distinguishing characteristics: latex is present in their sap, stems are erect, trailing or climbing according to the species, leaves are simple and arranged alternately around the stem, flowers are complete with superior pistil, five stamens and trumpet shaped corolla, the fruit is a capsule (Edmond, 1971). In North America, the family *Convolvulaceae* is economically important in that some members are troublesome weeds e.g. *Ipomoea cordatotriloba* Dennst., *I. hederacea* Jacq. (Clark et al., 1986), others are grown as

ornamentals e.g. *I. purpurea* (L.) Roth., others as rootstocks in sweetpotato flower induction e.g. *I. tricolor* Cav., and finally the sweetpotato itself is grown as a commercial and home garden commodity (Edmond, 1971).

2.1.2 Morphology, anatomy and physiology

Sweetpotato is propagated asexually and sexually, the asexual method is used by growers and research workers in the production of fleshy roots, while sexual propagation is used by breeders in the development of new varieties from seed (Edmond, 1971).

Young plants referred to as sprouts, cut-sprouts, slips or vines are used to establish commercial and home garden plantings in the USA (Edmond, 1971). Sprouts are entire plants which arise and are pulled from the bedded roots usually 6-8 inches long, with 4-6 physiologically active leaves. Cut-sprouts are essentially the above ground portions of the sprouts, the stems of individual plants are cut just above the planting media, and they range between 7-10 inches long. Vine cuttings are the terminal portions of the stem of plants growing in plant beds or in the field; usually stems are cut at the fifth or sixth node below the terminal. Cut-sprouts are mainly used for propagation in temperate regions, while vines are a common means in subtropical and tropical regions (Edmond, 1971). These young plants and vine cuttings are usually generated directly or indirectly from fleshy roots.

Generally, at the beginning of any producing season, fleshy roots are taken out of storage and bedded; the plants which develop from these roots are transplanted in the field. These plants in turn produce absorbing roots, stems, and leaves and fleshy roots. The fleshy roots are harvested and processed for human consumption or stored for production of young plants the following year. The sweetpotato in the USA is

propagated as an annual, as opposed to its perennial habit in the tropics (Edmond, 1971). Sweetpotato storage root formation involves distinct processes that include the induction of anomalous cambial cell formation, cell divisions, amyloplasts' biogenesis and starch accumulation. It is controlled by endogenous factors such as the hormones and was shown to be affected by external factors such as water availability, temperature, and nutrients. External and internal stimuli interact to determine whether an adventitious root becomes a fibrous root, a storage root or an intermediate structure, i.e., a pencil root in as early as 10 to 20 days after sprouts/slips are planted in the field (Firon et al., 2009; Villordon et al., 2012). The edible roots are either long and tapered, ovoid or round with skin color ranging from white, brown, purple or red, and the flesh color ranging from white, pale cream, orange or purple (Padmaja, 2009).

2.1.3 Distribution and economic importance

Sweetpotato is the seventh most important food crop in the world with an annual production of approximately 130 million tons grown on about 9 million hectares. It ranks third among root and tuber crops worldwide (FAO, 2008). It is mainly grown in developing countries which account for up to 95% of the world production. Asia is the leading producer accounting for 80% of the total world production with China producing 70%, though the major use is for livestock feed, Africa produces 15%, North America produces about 1.5%, while the rest of the world produces about 3.5% (FAO, 2008; Loebenstein, 2009).

In the USA, sweetpotato occupied an important diet of the poorer classes and of the slaves during colonial times in the southern states (Edmond, 1971). Sweetpotato was grown in Virginia, Carolina and New England as early as the 17th century (Gray, 1933).

Delaware, Maryland, Virginia and southern New Jersey emerged as the first commercial sweetpotato producing regions in 1909 (Edmond, 1971).

Sweetpotato has traditionally been used for consumption in the USA though it has varied widely depending on economic status especially during the 20th century (Smith et al., 2009). It was a very important part of diets that saved many from starvation during the depression era of the 1930s (Edmond, 1971). Currently, production is concentrated in the states of North Carolina, Mississippi, California and Louisiana. Louisiana is the fourth producer based on the most recent data with 6,070 hectares planted in 2011. North Carolina the leading producer had 26,304 hectares, Mississippi the second producer 8,903 hectares, while California the third producer had 7,486 hectares. Other producing states are Alabama, Arkansas, Florida, New Jersey and Texas with a combined acreage of 4,856 hectares (USDA-NASS, 2011). Of all the varieties released so far, Beauregard and Covington have had the greatest impact on the USA sweetpotato industry (Smith et al., 2009).

In China sweetpotato is the fifth largest staple crop after rice, wheat, maize and soybean, and is mainly used as food, feed and for industrial purposes (Zhang et al., 2009). Farmers in sweetpotato growing areas depend on it for income and food security. It played an important role in the 16th century when it was utilized to avoid starvation when other food crops failed. Major growing regions are concentrated in the Yellow River and Yangtze River valleys (Zhang et al., 2009).

Sweetpotato is one of the most widely grown roots crops in sub-Saharan Africa (SSA) covering about 2.9 million hectares (FAO, 2008). It is predominantly grown in small plots by poorer farmers tended mainly by women (Low et al., 2009). Production is

concentrated mainly in countries surrounding the Great Lakes region in Eastern and Central Africa with Uganda and Nigeria accounting for 33% of the total production in SSA. It tolerates less fertile soils and dry conditions, requires few inputs and less labor (Low et al., 2009). Rapid expansion in production during the last decade has been attributed to changes in crop patterns due to spread of cassava and banana diseases that have reduced acreage of the affected crops; unstable economies and increasing commercialization of production. However, lack of sustainable seed systems is one major constraint to improving sweetpotato productivity in SSA (Low et al., 2009)

2.1.4 Uses and nutritional value of sweetpotato

Sweetpotato is consumed mainly after cooking, baking or making fried chips. Roots can be canned or pureed for a longer shelf life. Sweetpotato based baby foods are commonly used as the first solid food for infants in most countries (Padmaja, 2009). The roots when cooked are either 'dry' or 'moist' depending on texture. The dry types are soft and mealy after cooking while the moist types are soft, watery and sticky (Rao et al., 1974).

Sweetpotato can be processed into various products that enhance shelf life such as dehydrated chips and flour, canned roots, frozen roots, fried products, sweetpotato puree, sweetpotato flakes, sweetpotato candies and sweetpotato pickles. The roots can also be used to process other secondary products such as noodles, sugar syrups, commodity chemicals from starch, non-alcoholic and alcoholic beverages and flour based products (Padmaja, 2009). The tender green vine shoots are utilized as human food in some countries (Villareal et al., 1979).

Sweetpotato roots are rich sources of starch and sugars, these carbohydrates account for up to 80 to 90% of the total dry matter (Padmaja, 2009; Truong et al., 1986). The crude protein content in most studied varieties ranges from 1.3 to 10% (Purcell et al., 1972). Sweetpotato roots are rich in carotenoids and vitamin C, and contain substantially good amounts of thiamin (B1) and riboflavin (B2). Beta-carotene (provitamin A) is the most abundant pigment especially in orange fleshed varieties. It is recognized as one of the best sources of vitamin A, and global efforts are being made to popularize the consumption of orange-fleshed varieties in countries and among populations, where vitamin A deficiency is a major problem (Padmaja, 2009). The roots are considered a highly functional, low calorie food with anti-diabetic effects, and have been reported to stabilize blood sugar levels and lower insulin resistance (Kusano and Abe, 2000; Ylonen et al., 2003).

2.2 Sweetpotato viruses

Vegetative propagation of sweetpotato from vines, sprouts or roots obtained from existing material makes it prone to accumulate pathogens, especially viruses that are inevitably transmitted with the propagation material to the newly planted fields leading to crop decline and poor root quality (Clark et al., 2012; Loebenstein et al., 2009). These viruses exist mainly as mixed infections, and their impact on yield and quality vary depending on the region, virus species and cultural practices (Clark et al., 2012). In temperate zones, the crop is affected by a complex of potyviruses and probably other unknown viruses that causes yield losses of about 20 to 40% (Clark and Hoy, 2006; Clark et al., 2012). These yield losses could probably be much higher were it not for the care taken to provide virus tested materials (Clark et al., 2012; Loebenstein et al., 2009). In

developing countries, for example in East Africa, lack of proper systems to provide virus tested materials to farmers, and the presence of the severe Sweet potato virus disease (SPVD) caused by synergism between SPFMV, and SPCSV can cause yield losses of 80 to 90% in high yielding genotypes (Karyeija et al., 1998). In China, yield losses of over 20% mainly due to SPFMV and *Sweet potato latent virus* (SPLV) were reported (Gao et al., 2000).

There are over 30 viruses and strains infecting sweetpotato worldwide, they belong to 9 families, *Bromoviridae* (1 virus), *Bunyaviridae* (1), *Caulimoviridae* (3), *Closteroviridae* (1), *Comoviridae* (1), *Flexiviridae* (1), *Geminiviridae* (15), *Luteoviridae* (1), and *Potyviridae* (9). Many of them are recently described DNA viruses belonging to families *Geminiviridae* and *Caulimoviridae*. Most of these viruses are associated with symptomless infections in sweetpotato and in some cases even in the indicator plant *I. setosa* (Clark et al., 2012).

2.2.1 Potyviruses (*Potyviridae*)

The potyvirus group in the family *Potyviridae* is the largest and economically most important of the 28 plant virus groups and families currently recognized (Shukla et al., 1994). Most potyviruses have narrow restricted host ranges, and infect a wide range of crops under varying environmental conditions (Shukla and Ward, 1989). They have been reported to infect 1,112 species of 369 genera in 53 plant families (Shukla and Ward, 1989). Their economic importance is highlighted by the fact that, in a recent survey of the ten most important filamentous viruses from each of the ten major world regions, 73% were potyviruses (Shukla and Ward, 1989). Potyviruses are transmitted in the non-persistent manner by many aphid species while some possible members have

fungus, mite or whitefly vectors. Members of the group investigated so far have all been found to induce characteristic "pinwheel" cytoplasmic inclusion bodies in infected plant cells (Shukla and Ward, 1989). Potyvirus particles are flexuous rods, 680-900 nm long and 11 nm wide, consisting of a single-stranded positive sense RNA with a genome of about 9.7 kb (Hull, 2002; Shukla and Ward, 1989). The most common sweetpotato potyviruses are *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato virus G* (SPVG), *Sweet potato virus 2* (SPV2), *Sweet potato latent virus* (SPLV) and *Sweet potato mild speckling virus* (SPMSV) (Loebenstein et al., 2009). Now that it is recognized as a distinct species, *Sweet potato virus C* (SPVC) is likely to be found as one of the most common sweetpotato viruses (Untiveros et al., 2010).

Sweet potato feathery mottle virus is the most widespread virus occurring in all sweetpotato growing regions worldwide (Clark and Moyer, 1988). The SPFMV genome is a filamentous particle (810-865 nm), single-stranded positive sense RNA, approximately 11.6 kb, and has a poly (A) tract at its 3' terminus and a genome-linked protein (VPg) at its 5' terminus (Brunt et al., 1996; Moyer and Cali, 1985; Sakai et al., 1997). Foliar symptoms include veinal chlorosis and feathering, and chlorotic spots with purple borders that appear mostly on older leaves of sweetpotato. Storage root symptoms may include russet crack, internal cork, shape deformities and surface discoloration depending on cultivar and virus strain present (Moyer and Salazar, 1989). SPFMV was originally differentiated into four strains: russet crack (RC), common (C), ordinary (O) and East African (EA) (Kennedy and Moyer, 1982; Kreuze et al., 2000). The RC and C strains are widely distributed, while EA seems to be most common in Africa, though it has also been reported in Peru, Spain and Easter Island (Gutiérrez et al., 2003; Kreuze et

al., 2000; Rännäli et al., 2009; Valverde et al., 2004). However, the C strain was recently separated into a distinct species, *Sweet potato virus C* (SPVC), due to its sequence divergence from the other three strains (Untiveros et al., 2010). SPFMV is non-persistently transmitted by aphids e.g. *Myzus persicae* (Sulzer), and *Aphis gossypii* Glover (Souto et al., 2003; Wosula et al., 2012). SPFMV can be mechanically transmitted to various *Ipomoea* spp such as *I. batatas*, *I. setosa*, *I. nil*, *I. incarnata* and *I. purpurea*, and some strains of *Nicotiana benthamiana*, *N. clevelandii*, *Chenopodium amaranticolor* and *C. quinoa* (Brunt et al., 1996). The virus is transmitted by grafting but not by seed or pollen or contact between plants (Loebenstein et al., 2009).

Sweet potato virus G was first reported in China, where it is also widespread (Colinet et al., 1998). It has subsequently been reported in the USA, Australia, Peru, Spain and Egypt (Ateka et al., 2004; Clark and Moyer, 1988; Clark and Hoy, 2006; IsHak et al., 2003; Souto et al., 2003; Tairo et al., 2006; Trenado et al., 2007; Untiveros et al., 2007). Recently, the virus was found in areas of the Pacific Ocean (Rännäli et al., 2008). It causes mottling in *I. nil*, and chlorotic spotting in *I. setosa* and *I. tricolor* (Souto et al., 2003). In sweetpotato it is mainly found in co-infections with SPFMV making it difficult to differentiate the symptoms caused by the two viruses (Clark et al., 2012). The virus is transmitted in a non-persistent manner by the aphids, *A. gossypii* and *M. persicae* (Souto et al., 2003; Wosula et al., 2012). The virus can also be mechanically transmitted to various *Ipomoea* spp. for example *I. cordatotriloba* (syn. *I. trichocarpa*), *I. hederacea*, *I. nil*, *I. setosa*, and *I. tricolor* (Brunt et al., 1996; Souto et al., 2003).

Sweet potato virus 2 (Synonyms: Sweet potato virus II, Sweet potato virus Y, Ipomoea vein mosaic virus) was first isolated and described from sweetpotato plants in

Taiwan (Loebenstein et al., 2009). SPV2 has been found in Australia, China, Portugal, South Africa, Zimbabwe, Spain, the USA, and Peru (Ateka et al., 2004; Souto et al., 2003; Tairo et al., 2006; Trenado et al., 2007; Untiveros et al., 2007). It induces chlorotic bands along sections of veins and discrete mosaic along the entire length of the veins in *I. setosa*, and vein mosaic in *I. nil* and *I. tricolor* (Ateka et al., 2007; Souto et al., 2003) but not in sweetpotato under greenhouse conditions (Ateka et al., 2004; Souto et al., 2003). It is mainly found in mixed infections with SPFMV and SPVG, although it spreads slowly in the field (Clark et al., 2012). The Taiwan isolate is non-persistently transmitted by *M. persicae* (Ateka et al., 2004), but the USA isolate has not been successfully transmitted by *A. gossypii* or *M. persicae* (Souto et al., 2003). It is mechanically transmitted to *I. nil*, *I. setosa*, *I. tricolor*, and several species of the genera *Chenopodium*, *Datura*, *Nicotiana*, and *Ipomoea* (Ateka et al., 2007; Loebenstein et al., 2009; Souto et al., 2003).

Sweet potato latent virus was first reported in Taiwan (Liao et al., 1979). It is widespread in China, and has also been reported from Korea, Indonesia, Japan, Philippines, Uganda, South Africa, India, Kenya and New Zealand (Loebenstein et al., 2009). The virus may cause mild chlorosis but in most cultivars the infection is symptomless (Loebenstein et al., 2009). It induces systemic mosaic and stunting in *N. benthamiana*; systemic pin-prick chlorotic lesions in *N. clevelandii*; brown necrotic local lesions in *C. quinoa* and *C. amaranticolor*, and systemic mottle in *I. setosa* (Loebenstein et al., 2009). Isolates from Japan and China were transmitted by *M. persicae* (Usugi et al., 1991). It can be transmitted by mechanical inoculation and grafting, but not through seed (Loebenstein et al., 2009).

Sweet potato mild speckling virus was first discovered in Argentina from plants with Chlorotic dwarf complex disease that also included SPFMV and SPCSV (Di Feo et al., 2000). The virus has also been found in China, Egypt, India, Indonesia, New Zealand, Nigeria, Peru, Philippines, and South Africa (Loebenstein et al., 2009). It induces occasional chlorotic speckling in sweetpotato; vein clearing, blistering, leaf deformation and mosaic in *I. nil* and *I. setosa*; vein clearing, and reduction, deformation and down rolling of leaves in *N. benthamiana*, and local infections in *C. quinoa* and *N. tabacum* (Di Feo et al., 2000; Loebenstein et al., 2009). It is transmitted by *M. persicae* in a non-persistent manner, and through mechanical inoculation and grafting (Di Feo et al., 2000; Loebenstein et al., 2009).

2.2.2 Ipomoviruses (*Potyviridae*)

The ipomovirus group in the family *Potyviridae* consists of flexuous rods 800-950 nm long with a genome of 10.8 kb, and induces pinwheel inclusions in host cytoplasm (Hollings et al., 1976; Hull, 2002). Known vectors of viruses in this group are whiteflies of the species *Bemisia tabaci* Genn. (Hull, 2002). *Sweet potato mild mottle virus* (SPMMV) is so far the only sweetpotato infecting virus found in this group (Clark et al., 2012).

Sweet potato mild mottle virus was first described as a whitefly-borne virus from Kenya, Uganda and Tanzania (Hollings et al., 1976). It has so far been reported in South Africa, Indonesia, China, Philippines, India, Papua New Guinea and New Zealand (Loebenstein et al., 2009). In Africa it is the third most prevalent virus after SPFMV and SPCSV (Ateka et al., 2004; Mukasa et al., 2003; Tairo et al., 2005). The virus was transmitted to plants in 14 families (Hollings et al., 1976). It induces leaf mottling and

stunting in sweetpotato, though some cultivars remain symptomless. In diagnostic hosts, it causes vein clearing, leaf puckering, mottling and distortion in *N. tabacum* and *N. glutinosa*; local lesions in *C. quinoa*, and conspicuous systemic vein necrosis in *I. setosa* (Loebenstein et al., 2009). The virus is transmitted by *B. tabaci* in a semi-persistent manner, by grafting and mechanical inoculation (Loebenstein et al., 2009).

2.2.3 Criniviruses (*Closteroviridae*)

Criniviruses have flexuous filamentous particles that have two modal lengths, 700-900 nm and 650-850 nm and about 12 nm in diameter. The genomic nucleic acid is bipartite, linear, positive sense ssRNA. They have a narrow host range, mostly phloem limited and cause yellowing type symptoms. They are transmitted in a semi-persistent manner by whiteflies, *Bemisia* spp and *Trialeurodes* spp (Hull, 2002). *Sweet potato chlorotic stunt* (SPCSV) is so far the only crinivirus known to infect sweetpotato (Clark et al., 2012).

Sweet potato chlorotic stunt virus (Syn. Sweet potato sunken vein virus) was first described in Nigeria (Schaefer and Terry, 1976). It exists in two geographically distinct strains, the SPCSV East African (EA) strain, and the SPCSV West African (WA) strain (Vetten et al., 1996). SPCSV-EA has been reported from Madagascar, Kenya, Peru, Uganda and Zambia (Ateka et al., 2004; Gibson et al., 1998; Gutierrez et al., 2003), while SPCSV-WA from Egypt, Israel, Nigeria, Niger, Spain, Peru and USA (Abad et al., 2007; Carey et al., 1999; Fenby et al., 2002; Gutiérrez et al., 2003; IsHak et al., 2003; Valverde et al., 2004). The single-stranded positive-sense RNA is bipartite and comprises one of the largest genomes among single-stranded plant viruses. Genomic RNA 1 is 9,407 and RNA 2 is 8,223 nucleotides with RNA 2 having the five-gene

module typical of the family *Closteroviridae* including the heat shock protein homologue (Hsp70h) and the major coat protein (Cuellar et al., 2008; Kreuze et al., 2002). The virus may cause interveinal chlorosis and interveinal purpling on older leaves in sweetpotato. It causes mild interveinal chlorosis, general chlorosis, brittle leaves and stunting in *I. setosa*; and leaf distortion, stunting and chlorosis in *I. nil* (Loebenstein et al., 2009). SPCSV is the critical synergizing component in devastating disease complexes such as Sweet potato virus disease (SPVD) and Sweet potato chlorotic dwarf in which it suppresses host resistance of the sweetpotato enabling other viruses to replicate more efficiently and cause symptoms much more severe than in single infections. In addition to the well-known synergism with SPFMV, SPCSV can also enhance infections by SPVG, SPV2, SPMSV, SPLV and SPMMV (Clark et al., 2012; Loebenstein et al., 2009). The virus is transmitted in a semi-persistent manner by the whitefly *B. tabaci* biotype B, *Trialeurodes abutilonea* (Haldemann) and *Bemisia afer* (Priesner and Hosny) (Gamarra et al., 2010; Ng and Falk, 2006, Schaefer and Terry, 1976; Sim et al., 2000; Valverde et al., 2004). It is also graft transmissible, but not through mechanical inoculation (Loebenstein et al., 2009).

2.2.4 Begomoviruses (*Geminiviridae*)

Plant viruses in this group have circular single stranded DNA genomes contained in geminate virus particles. Most begomoviruses have two DNA molecules and are transmitted by whiteflies (Hull, 2002).

Sweet potato leaf curl virus was first reported from Japan and Taiwan (Liao et al., 1979; Shinkai, 1979). The virus has so far been reported from Brazil, China, Mexico, Korea, Puerto Rico, Peru, Kenya and USA (Fuentes and Salazar, 2003; Loebenstein et al.,

2009; Lotrakul et al., 1998; Miano et al., 2006). The circular ssDNA genome consists of 2,828 nucleotides, six open reading frames, and an intergenic region containing a conserved stem-loop motif typical of geminiviruses (Lotrakul and Valverde, 1999). The virus causes transient upward curling of leaves and vein swelling on young leaves of sweetpotato plants. It causes leaf curl in *I. nil*, *I. setosa* and *N. benthamiana*, and yellow vein symptoms in *I. aquatica* (Lotrakul et al., 1998). The virus is transmitted by *B. tabaci* biotype B in a persistent manner, and by grafting but not through mechanical or seed inoculation (Valverde et al., 2004).

2.3 Aphids as vectors of sweetpotato viruses

Plant viruses are obligate parasites, and a majority of them about 70% depend on vectors for their transmission and survival. Insects, mites, nematodes and protists are known to vector plant viruses, with insects being the most common vectors (Brunt et al., 1996; Nault, 1997).

Aphids account for about 50% of the insect vectored plant viruses (Nault, 1997). A majority of the vectors belong to the Subfamily *Aphidinae* (Order: Hemiptera). Aphid vectors are also found in nine other subfamilies, but they account for only a very small proportion of those that are known to transmit viruses (Blackman and Eastop, 2000). Aphids are successful vectors of plant viruses because they possess three important attributes that favor dispersion of viruses. They are polyphagous i.e. they feed on a wide range of plant hosts; they reproduce parthenogenetically facilitating rapid population build up; and they possess needle-like piercing and sucking mouth parts referred to as stylets that are capable of piercing plant cells and delivering viruses in the host (Ng and Perry, 2004). The potential of an aphid as a vector depends on feeding behavior and host

plant selection (Ng and Perry, 2004). There are three modes through which aphids transmit viruses from plants: non-persistent, semi-persistent and persistent transmission (Nault, 1997). Non-persistent transmission involves rapid acquisition and inoculation of the virus by aphids usually in a matter of seconds. Aphid stylets do not usually penetrate beyond epidermal cells during virus acquisition and inoculation probes. Once acquired, they are retained by the vector for very few hours (Nault, 1997; Nault and Bradley, 1969). Aphid vectors acquire and transmit non-persistent viruses without necessarily colonizing the infected plants due to their feeding habit that involves sap sampling/probing that lasts usually for less than a minute (Powell et al., 2006). SPFMV, SPVG and SPV2 are examples of sweetpotato viruses transmitted non-persistently (Kennedy et al., 1962; Souto et al., 2003; Wosula et al., 2012). Semi-persistent viruses may be acquired and transmitted within minutes or hours. Some are found in all plant cells, while the majority these viruses are phloem limited (Hull, 2002). The time required for acquisition and transmission may depend on how long the aphid takes to reach the phloem. Once acquired, they can be retained for days or probably weeks (Gray and Banerjee, 1999; Ng and Falk, 2006). Persistent viruses require longer acquisition and inoculation times, probably hours to days and latent periods that may range from one day to weeks. They are phloem limited and therefore transmitted almost solely by insects that colonize the affected plant hosts. Once acquired, these viruses are associated with the vector for the rest of its life (Gray and Banerjee, 1999; Ng and Perry, 2004).

2.3.1 Distinguishing features and classification

Aphids are very successful insect pests and vectors of plant viruses because of their complex life cycles and close association with their host plants, their ability to reproduce both asexually and sexually, and their polyphenism (ability to form winged and wingless aphids) (Dixon, 1998). There are about 4000 aphid species in temperate regions where one in every four plant species can be infested (Dixon, 1998).

Aphids are small (1-10 mm), soft-bodied plant sucking insects. In most cases, several or all generations are composed of asexually reproducing females (parthenogenesis). Usually eggs of parthenogenetic females begin development immediately after ovulation; a nymph can have embryos developing within it that also have embryos (telescoping of generations). Parthenogenesis and telescoping of generations are two attributes that favor aphids to achieve rapid population increase rates. Aphids also exhibit polyphenism, a phenomenon in which they exist in different morphs mainly as winged aphids (alate) and wingless aphids (apterae). The most obvious diagnostic morphological features are the wings, abdomen, antennae, cauda and siphunculi (Dixon, 1998). Aphids belong to class insecta, order Hemiptera and are classified into nine major families with *Aphididae* being the largest family (Dixon, 1998).

2.3.2 Host specificity, location and recognition

Most aphids are autoecious, living on one or few species of a particular genus of plants. Heteroecious species (about 10% of aphids) spend winter and spring on primary host plants, and summer on secondary host plants not necessarily related to the primary host. For example the green peach aphid *Myzus persicae* overwinters on peach (*Prunus*

persica L.), but exploits a wide range of secondary host plants during summer (Dixon, 1998).

Aphids locate their host plants by responding to visual cues, especially yellow colors. They use color as an indicator of the nutritional status of the plant. The young and senescing leaves that appear yellower are more nutritious than mature leaves (Kennedy, 1961). After locating and landing on a host plant, aphids walk on the surface testing it with their antennae and probing it with their mouth parts. While walking and probing the plant surface, an aphid obtains information about physical properties and chemistry of the plant. This initial investigation of the plant surface involves little or no stylet penetration, but enables the aphid to sense plant suitability within one minute (Dixon, 1998). Once an aphid settles, it penetrates the plant with its stylet. The black bean aphid (*Aphis fabae* Scopoli) takes approximately 40 minutes to reach the phloem of its host plant, but this period may be longer for aphids that feed on phloem elements situated deep within woody tissues of plants (Dixon, 1998).

2.3.3 Feeding behavior

A majority of the aphids feed on phloem sap of plants, which they obtain using their feeding mouth parts (stylets). Aphids have antennae bearing many sensilla which are used in chemoreception or gustation and perception of the leaf surface (Park and Hardie, 2004). Aphids scan surfaces of potential host plants using the tips of the proboscis which have tactile receptors that enable them to detect the contours of veins (preferred feeding sites) (Tjallingii, 1978). They then probe into the plant with their mandibular and maxillary stylets which together form a hollow needle-like structure. During the penetration process into the plant tissues, aphids normally secrete a salivary

sheath that encases the stylet. This sheath protects the delicate stylets and enables aphids to control the direction of the probe by restricting bending except at the apex of the stylets (Pollard, 1973). The salivary sheath normally ends in the phloem indicating that aphids feed on the contents of the sieve elements (Pollard, 1973). Aphids that reach the phloem of resistant cultivars of their host plant tend to cease feeding shortly after the phloem is penetrated. Also aphids feeding on non-host plants initially ingest phloem sap at normal rates, but suddenly cease feeding, withdraw their stylets and leave the plant (Dixon, 1998). These observations have led to suggestions that phloem sap of resistant or a non-host plant is nutritionally unsuitable (Dixon, 1998).

The level of soluble nitrogen in host plants determines the growth and reproduction of aphids (Dixon, 1998). Plants that are growing, flowering or senescing usually have more nitrogen in their phloem sap because nutrients are being translocated as opposed to plants with mature leaves or that have ceased growing. Therefore, the food of aphids shows marked variations in quality in space (different parts of the plant) and time (different stages in the seasonal growth cycle of the plant) (Dixon, 1998).

2.3.4 Flight patterns of aphids

The daily flight of aphids depends on the rate of development of the winged adults (Johnson et al., 1957). Flight is inhibited at night by low light intensity and occasionally during the day by low temperatures. Two daily flight peaks were observed, the first was during the morning (aphids that matured overnight and prevented from flying due to darkness and low temperatures), and the second was in the afternoon (aphids that molt in the morning and complete their teneral development by the afternoon) (Johnson et al., 1957).

Seasonal flight activity of aphids depends on the species, some may have single flight peaks e.g. the green spruce aphid [*Elatobium abietinum* (Walker)], two flight peaks e.g. the hop aphid [*Phorodon humuli* (Schrank)] or three flight peaks e.g. bird cherry-oat aphid [*Rhopalosiphum padi* (L.)]. The spring and the fall peaks of aphid flight activity of host alternating species represent aphids leaving and returning to the primary host, while the summer flight activity represents aphid redistribution between secondary host plants (Dixon, 1998). The flight behavior of aphids that make up the spring, summer and fall flights differ. In experiments in flight chambers, fall migrants tend to have greater initial rates of climb and spend longer in the migratory phase than the spring and summer migrants (David and Hardie, 1988). In host alternating species the seasonal pattern of flight activity reflects the optimum time for host transfer between primary and secondary host plants (Dixon, 1998).

2.3.5 Electronic monitoring of aphid probing/feeding behavior

Feeding behaviors of piercing and sucking insects like aphids usually occur inside the plant tissues and are not easily observable. Special techniques are therefore required to study the feeding behavior of these insects (Walker, 2000). McLean and Kinsey (1964) made the most significant advance towards developing specialized techniques for studying hemipteran feeding behavior when they invented the electronic feeding monitor. This was later modified and improved (Tjallingii, 1985) and is currently referred to as electrical penetration graph (EPG) monitor. There are two types of EPG monitors, those that use AC (alternating current) circuitry and are referred to as AC EPG systems (Backus and Bennett, 1992), and those that use DC (direct current) circuitry and are referred to as DC EPG systems (Tjallingii, 1985). All EPG monitors (AC and DC

systems) measure changes in electrical resistance in the plant and probing insect. In addition to changes in resistance, the DC system also measures changes in voltage that originate in the plant and probing insect (Tjallingii, 1978). The basic principle behind EPG monitors according to Walker (2000) is: the EPG monitor has two electronic components, a voltage source and an input resistor that are electrically connected to each other with two receptacles; output one connected directly to the voltage source and the input one connected to the input resistor. The use of EPG to study hemipteran insects involves making the insect and the plant part of the circuit. This is done by connecting a wire from the output receptacle to the plant and another wire from the input receptacle to the insect. The output wire makes electrical contact with the plant by connecting to a stiff copper wire inserted in the potting mixture. The input receptacle makes contact with the insect by connecting to a very thin gold wire (2.5-25 μm) glued to the insect's dorsum using silver paint. The gold wire is attached to a 2 cm long silver wire soldered on a copper pin (Walker, 2000).

Aphid behaviors recorded by EPGs include probing (stylets inserted inside the plant tissues) and non-probing. Within probing, different activities can be recognized in EPGs as distinct electrical waveforms or waveform patterns. The aphid EPGs from DC systems, the stylet pathway contains waveforms A, B, C and potential drops (pds), a xylem phase represented by waveform G, and a phloem sieve element phase including waveforms E1 and E2 (van Helden and Tjallingii, 2000).

2.4 Whiteflies as vectors of sweetpotato viruses

Like aphids, whiteflies have piercing and sucking mouth parts (stylets) that are favorable to vectoring of plant viruses. They belong to the family *Aleyrodidae* of the

order Hemiptera. About 1300 whitefly species in over 120 genera have been described (Mound and Halsey, 1978), but relatively few transmit plant viruses. Only whiteflies in the *Bemisia* and *Trialeurodes* genera are virus vectors. In the genus *Bemisia*, only *B. tabaci* and *Bemisia afer* have been shown to be vectors whereas in the *Trialeurodes* genus, *Trialeurodes vaporariorum* (Westwood), *Trialeurodes abutilonea* and *Trialeurodes ricini* (Misra) transmit viruses (Gamarra et al., 2010; Jones, 2003). Three species of whiteflies, *Bemisia tabaci* (sweetpotato whitefly), *Trialeurodes vaporariorum* (greenhouse whitefly) and *T. abutilonea* (banded-winged whitefly), are known to transmit plant viruses. Several biotypes of *B. tabaci* are known to transmit begomoviruses in a persistent-circulative manner, while *T. abutilonea*, *T. vaporariorum*, and *B. tabaci* vector closteroviruses and criniviruses semi-persistently (Brown and Bird, 1992; Wisler et al., 1998).

2.5 Wild hosts of sweetpotato viruses

Sweetpotato viruses have been detected in wild plants mainly of the morning glory family (Clark et al., 1986; Tugume et al., 2008). In Louisiana, USA, SPFMV has been found infecting several *Ipomoea* species, for example, *Ipomoea cordatotriloba* (syn. *I. trichocarpa*), *I. hederacea*, *I. hederifolia*, *I. lacunosa* and *I. wrightii* (Clark et al., 1986). SPFMV has also been detected in 24 wild plant species of family *Convolvulaceae* occurring in different regions in Uganda (Tugume et al., 2008). In Syria, a recent survey indicates 19 species belonging to family *Chenopodiaceae* and *Convolvulaceae* may be natural hosts of SPFMV (Akel et al., 2010). SPMMV was detected in 21 wild species, all of which were previously unknown natural hosts in Uganda (Tugume et al., 2010). SPLCV has been detected naturally infecting *I. hederacea*, *I. wrightii*, *I. setosa* and *I.*

tenuissima in the USA, and was experimentally transmitted by whiteflies to 38 species in the genus *Ipomoea* (Ling et al., 2011). Lack of spatial and temporal separation between populations of wild host plants and cultivated sweetpotato is an important aspect in enhancing virus exchange between natural and agro-ecosystems considering the similarity in genotypes of viruses found in both systems (Clark et al., 2012).

2.6 Detection methods for sweetpotato viruses

Reliable techniques for rapid detection and identification of plant viruses are essential for their timely management. Sweetpotato viruses are challenging to detect due to low titers and uneven virus distribution, presence of high concentration of inhibitors in sweetpotato plants that interfere with serological or PCR-based methods, occurrence as mixed infections, and diverse strains (Clark et al., 2012; Valverde et al., 2007).

Traditionally, sweetpotato plants are indexed for the presence of viruses by grafting to the Brazilian morning glory *I. setosa* (Clark et al., 2012). Other host plants that are recommended and used for indexing include *I. nil*, *Nicotiana benthamiana*, *N. clevelandii* and *Chenopodium quinoa* (Moyer and Salazar, 1989). However, these biological indexing procedures require considerable time, labor, and greenhouse space and do not reveal the identity of viruses present (Clark et al., 2012; Valverde et al., 2007).

Serological methods such as the well-known and widely used enzyme-linked immunosorbent assay (ELISA) are used for detection of sweetpotato viruses preferably after indexing on *I. setosa*. A membrane immuno-binding assay also known as nitrocellulose membrane ELISA (NCM-ELISA) has been used with success to detect several sweetpotato viruses (Abad and Moyer, 1992; Clark et al., 2010; Gutierrez et al. 2003; Souto et al. 2003). Detection kits using this technique have been developed by the

International Potato Center and are very practical, particularly in developing countries where the use of other methods is limited by the available resources (Valverde et al., 2007).

Quantitative PCR is one of the most recently used techniques to detect, quantify, and/or identify sweetpotato viruses. It is sensitive and accurate compared to most conventional methods. In this method, the amplification of mRNA or coding sequence of a host gene needs to be included as an internal control in detection of RNA and DNA viruses, respectively. Sweetpotato genes including 18S and 26S rRNA genes, the cytochrome oxidase (*COX*) gene and plant mitochondrial NADH dehydrogenase (*Nad5*) gene have been used as internal controls in the quantitative PCR assays to normalize differences in RNA and DNA concentrations between samples (Lee and Chang, 2006; McGregor et al., 2009; Mukasa et al., 2006; Wasswa et al., 2011). Several viruses for example SPCSV, SPFMV, SPLCV, SPMMV, SPVG and SPV2 have been detected and quantified directly from sweetpotato plants using this method (Kokkinos and Clark, 2006; McGregor et al., 2009; Mukasa et al., 2006).

Conventional PCR techniques have also been widely used for sweetpotato virus detection. Reverse transcription PCR (RT-PCR) with universal degenerate primers and strain specific primers has allowed detection and characterization of many potyviruses in sweetpotato (Ateka et al., 2004; Colinet et al., 1997; Gibbs and MacKenzie, 1997; Souto et al., 2003). SPCSV can be detected with universal primers that amplify a portion of the gene that encodes the heat shock protein homologue present in members of family *Closteroviridae* (Sim et al., 2000). Begomoviruses have been detected and identified in tissue culture plantlets, sweetpotato plants, and *Ipomoea* indicator plants using PCR with

generic and virus specific primers (Banks et al., 1999; Li et al., 2004; Lotrakul et al., 2002; Lozano et al., 2009; Wasswa et al., 2011).

2.7 Yield loss due to sweetpotato viruses

Sweetpotato root yields differ greatly in different growing regions. The average yield in African countries is about 7.02 tons/ha, in Asia 12.41 tons/ha, in South America 10.74 tons/ha, while in the USA the average is 20.1 tons/ha (Loebenstein et al., 2009). These differences in yield are mainly attributed to quality of propagation material and fertility, which is usually collected in the previous season from a farmer's fields. In most cases the propagation material is infected with several viruses that eventually have a negative impact on yield (Loebenstein et al., 2009). Gao et al. (2000) reported yield losses of up to 20% attributed to infection with SPFMV and SPLV in China. Sweet potato virus disease (SPVD) caused by synergism between SPFMV and SPCSV causes yield losses within the range of 50 to 90% in East Africa (Gibson et al., 1997; Karyeija et al., 1998). Under experimental conditions, SPVD caused root yield losses by 56 to 90% in Cameroon (Ngeve and Bouwkamp, 1991); 78% yield reduction in Nigeria (Hahn, 1979); about 50% yield reduction in Israel (Milgram et al., 1996); and 65 to 72% yield reduction in Peru (Gutiérrez et al., 2003). In temperate zones, potyviruses and probably other unknown viruses cause yield losses in the range of 20 to 40% (Clark and Hoy, 2006).

2.8. Management of sweetpotato viruses

Sweetpotato virus management attempts are recent, and mainly involve use of clean virus-tested planting material or resistant cultivars. Meristem-tip culture techniques have been used to produce plants free of detectable viruses. In the USA sweetpotato

foundation 'seed' program was primarily for reduction of mutations. However, in the 1960s virus testing seed programs were initiated in California as a means of managing russet crack disease (Dangler et al., 1994). Virus testing has been integrated in foundation seed programs in other states for the last 10 to 20 years. Although clean seed technologies have been implemented in many temperate countries, they are not yet widely adopted in tropical countries (Clark et al., 2012; Villordon et al., 1996). An extensive survey on the benefits of using virus tested planting material was carried out in Shandong, China, and revealed that up to 80% of the farmers adopted the technology and overall there was a yield increase of about 30% (Fuglie et al., 1999). In the USA, foundation seed programs provide growers with a small stock of clean planting material which they must increase on their farms in order to produce enough material to plant succeeding crops (Bryan et al., 2003). Although there is a yield and quality benefit from use of early generation propagation material (Bryan et al., 2003; Carroll et al., 2004; Clark et al., 2010), these materials are often rapidly re-infected with viruses during the first year in the field (Clark et al., 2010). Breeding for resistant cultivars might be a better strategy for management of sweet potato viruses, especially in Africa where lack of resources and the nature of farming systems are limiting factors for use of virus tested planting material. Breeding for resistance to SPVD has already been initiated in Uganda and is combined with breeding for other desirable traits such yield earliness and acceptable culinary quality (Karyeija et al., 2000; Mwangi et al., 2002). Cultural practices such as selection of disease free planting material, roguing of diseased plants, control of wild *Ipomoea* spp, isolating new crops from old ones, use of barrier crops, or intercropping may minimize the impact of viral diseases (Gibson and Aritua, 2002).

CHAPTER 3: EFFECT OF HOST PLANT, APHID SPECIES, AND VIRUS INFECTION STATUS ON TRANSMISSION OF SWEET POTATO FEATHERY MOTTLE VIRUS¹

3.1 Introduction

Sweetpotato [*Ipomoea batatas* (L.) Lam.] is the seventh most important food crop in the world with an annual production of approximately 130 million tons, ranking third among root and tuber crops worldwide (FAO, 2005). It is increasing in demand in the USA given its perception as a nutritious food with more processed products becoming available. Sweetpotato is an important carbohydrate source, especially in Africa, where it provides household food security, because it persists well in the soil as a famine reserve crop and performs well in marginal soils (Byamukama et al., 2004; Clark et al., 2010). Sweetpotato is vegetatively propagated and is prone to accumulate viruses which cause cultivar yield decline and reduce storage root quality (Clark et al., 2002; Clark and Hoy, 2006). The most common sweetpotato viruses in the USA are members of the family *Potyviridae* and the genus *Potyvirus*: *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato virus G* (SPVG) and *Sweet potato virus 2* (SPV2, synonym = *Ipomoea vein mosaic virus*) (Clark and Moyer, 1988; Souto et al., 2003; Clark et al., 2012).

Sweetpotato potyviruses are restricted primarily to the family *Convolvulaceae*, transmitted by aphids, and occur commonly as mixed infections in the field (Byamukama et al., 2004; Moyer and Salazar, 1989; Stubbs and McLean, 1958).

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SPFMV is the most common sweetpotato virus and occurs in all sweetpotato growing areas including the USA (Clark and Moyer, 1988). Four strains were originally differentiated: russet crack (RC), common (C), ordinary (O) and East African (EA) (Kennedy and Moyer, 1982; Kreuze et al., 2000). The RC and C strains are widely distributed, while EA seems to be most common in Africa, though it has also been reported in Peru, Spain and Easter Island (Kreuze et al., 2000; Gutiérrez et al., 2003; Valverde et al., 2004; Rännäli et al., 2009). However, the C strain was recently separated into a distinct species, *Sweet potato virus C* (SPVC), due to its sequence divergence from the other three strains (Untiveros et al., 2010). Single infections of SPFMV or mixed infections with SPVG and SPV2 in sweetpotato cv. Beauregard usually show mild chlorotic spotting and veinal chlorosis or no symptoms, and cause little or no yield loss (Kokkinos and Clark, 2006; Clark et al., 2010). However, naturally infected sweetpotato plants (i.e. grown in the field for several years) which test positive for these three potyviruses and negative for other known sweetpotato viruses, may display distinct symptoms accompanied with yield reduction (Clark and Hoy 2006; Clark et al., 2010), possibly indicating the presence of other unknown viruses.

Mixed virus infections can positively or negatively impact transmission rates and alter patterns of virus spread (Rochow, 1972). Mixed infections could be better sources of inoculum for some viruses, while for others transmission rates are greater from single infections (Rochow, 1972). In Louisiana, after one generation in the field, plants are commonly infected with SPFMV, but by the fourth generation, plants usually test positive for SPFMV (100%), SPVG (50 to 70%) and SPV2 (25 to 30%) (Clark et al.,

2010). Since SPVC was not recognized as a distinct species until recently, data are not available for its occurrence.

Experimental host range studies suggest that other wild virus hosts are potential sources of inoculum, (Clark and Moyer, 1988) mainly morning glory plants in the genus *Ipomoea*, family *Convolvulaceae* (Loebenstein et al., 2009; Tugume et al., 2008). In Louisiana, the morning glories *Ipomoea cordatotriloba* Dennst. (synonym *I. trichocarpa* Ell.) and *I. hederacea* Jacq. are common in and around sweetpotato fields, and *I. hederifolia* L., *I. lacunosa* L., and *I. wrightii* (Wall.) are also known wild hosts of SPFMV (Clark et al., 1986).

SPFMV is transmitted by several aphid species (Stubbs and McLean, 1958) in a non-persistent manner (Kennedy et al., 1962; Kennedy and Moyer, 1982). Aphid vectors acquire and transmit potyviruses to susceptible plants without necessarily colonizing the infected plants due to their sap sampling/probing feeding habit (Powell et al., 2006). These aphids acquire the virus in as short as 3 to 35 seconds (McLean, 1959), and retain the virus for a short period, normally less than two hours (Ng and Falk, 2006). Efficient vectors of SPFMV are the aphid species *M. persicae* and *A. gossypii* (Stubbs and McLean, 1958; Byamukama et al., 2004). *Myzus persicae* occasionally colonizes sweetpotato in Louisiana; while *A. gossypii* has been found on *I. hederacea* (Clark, C. A. personal communication).

Potyviruses are detected in sweetpotato using various combined methods. Since they exist in very low titers in sweetpotato (Kokkinos and Clark, 2006), graft indexing on *I. setosa* is traditionally used to detect its presence and subsequent testing using other techniques. The most widely used serological method (Clark et al., 2012) is a membrane

immune-binding assay known as nitrocellulose membrane ELISA (NCM-ELISA).

Reverse transcription PCR (RT-PCR) and quantitative reverse transcription PCR (qRT-PCR) are also used for detection and quantification (Clark et al., 2012; Kokkinos and Clark, 2006)

The aim of this study was to determine the transmission efficiency of SPFMV-RC, the most prevalent potyvirus strain in Louisiana, from potential sources which may occur in the field. We tested transmission efficiency from the predominant Louisiana sweetpotato cultivars, Beauregard and Evangeline, the two most common morning glory plants in or near sweetpotato fields, *I. hederacea* and *I. cordatotriloba*, and compared single SPFMV infections with representative natural mixed infections using the aphid vectors *M. persicae* and *A. gossypii*.

3.2 Materials and methods

3.2.1 Host plants

Ipomoea nil cv. Scarlet O'Hara (SOH) was used as the test plant in all studies. The following *Ipomoea* species were used as SPFMV acquisition sources: sweetpotato cvs. Beauregard and Evangeline, *I. cordatotriloba* (cotton morning glory) and *I. hederacea* (ivy-leaf morning glory). Sweetpotato plants were derived from virus-tested mericlones maintained by nodal propagation in tissue culture at the LSU AgCenter Department of Plant Pathology and Crop Physiology. *Ipomoea cordatotriloba* and *I. hederacea*, were established from seeds harvested from single plants that were grown in the greenhouse. All plants were grown in the greenhouse under wide temperature (10-32°C) and humidity (21-98%) ranges, in 10 cm diameter clay pots containing autoclaved soil mix consisting of 1 part river silt, 1 part sand, 1 part Jiffy-Mix Plus (Jiffy Products of

America Inc., Norwalk, OH) and Osmocote 14-14-14 (Scotts-Sierra Horticultural Products Company, Marysville, OH) at 3.5 g/pot. Plants were not sprayed with insecticide. Establishment of sweetpotato plants from virus tested mericlones, and morning glory plants from seeds ensured they were virus free before being used in studies.

3.2.2 Virus inoculum

Two inoculum sources were used in our transmission studies: plants infected with the russet crack strain of SPFMV (SPFMV-RC, isolate 95-2) maintained in SOH in the greenhouse by repeated mechanical inoculation, and a naturally mixed infected sweetpotato cv. Beauregard (B 14, G-7) that was grown in fields in North Carolina for seven years and provided by G. C. Yencho (Dept. Horticultural Sciences, North Carolina State University, Raleigh). B 14, G-7 was tested using NCM-ELISA after grafting on *I. setosa*, RT-PCR and q RT-PCR and found to be infected with SPFMV, SPVG and SPV2, but tested negative for *Sweet potato mild mottle virus*, *Sweet potato latent virus*, *Sweet potato chlorotic fleck virus*, *Sweet potato mild speckling virus*, *Sweet potato leaf curl virus*, *Sweet potato chlorotic stunt virus*, *Sweet potato collusive virus*, and *Cucumber mosaic virus*. However, the possibility that it was infected by viruses not yet recognized in sweetpotato cannot be eliminated.

3.2.3 Aphid colony

Aphis gossypii was collected from cotton at the LSU AgCenter Macon Ridge Research Station, Winnsboro, LA in 2006, while *M. persicae* was collected from an unknown host in 2004. Aphid colonies were established from single aptera and maintained under laboratory conditions in screened cages at room temperature (20-22°C)

and a 16L: 8D photoperiod. *Aphis gossypii* was reared on cotton plants (*Gossypium* spp) cv. Stoneville 474, while *M. persicae* was reared on mustard plants (*Brassica cretica* L.) cv. Tendergreen, neither of which has been described as a host for sweetpotato viruses. Plants were grown in the greenhouse under wide temperature (10-32°C) and humidity (21-98%) ranges, were fertilized on a weekly basis with NPK 20-20-20 (Scotts-Sierra Horticultural Products Company) and kept free of insecticides. A cohort of 5 to 10 aphids was placed on fresh plants using a paint brush to establish new colonies every 2 to 3 weeks.

3.2.4 NCM-ELISA assays

Leaf tissue was collected from symptomatic plants and assayed by nitrocellulose membrane-enzyme linked immunosorbent assay (NCM-ELISA) (Clark et al., 2010) using antisera produced to the russet crack strain of SPFMV provided by J.W. Moyer (North Carolina State University, Raleigh), or antisera to isolates from Louisiana of SPVG and SPV2 provided by S. Fuentes (International Potato Center, Lima, Peru). A small piece (~ 1 cm²) was collected from a lower, middle, and upper leaf of each plant, combined, placed in a “Universal” extraction bag (Article No. 430100; BIOREBA, Reinach BL 1, Switzerland), and homogenized using a HOMEX6 homogenizer (BIOREBA) in 8 ml of Tris-buffered saline (TBS = 0.02 M Tris base, 0.50 M NaCl) pH 7.5 containing 0.2% sodium sulfite (Na₂SO₃). Two ml of the extracted tissue were transferred to a 2 ml microfuge tube and allowed to stand for 30 to 45 minutes at room temperature. The clarified sap extract (50 µl) was blotted onto a TBS buffer-saturated nitrocellulose membrane and air-dried for 20 to 30 minutes. All the incubations and washings were done at room temperature in a shaker with gentle agitation (50 rpm for incubations and

100 rpm for washings) using reagents and procedures according to Clark et al. (2010). The first antibody (polyclonal specific to SPFMV, SPVG or SPV2) was cross absorbed in healthy *I. setosa* extract (1 g tissue homogenized in 25 ml of TBS containing 2 g·per liter sodium sulfite, 20 g·per liter Carnation condensed milk (Nestlé USA, Inc., Solon, OH), and 0.2 g·per liter sodium azide – pH 7.5) for 45 minutes at 37°C and was then added to the membranes and incubated overnight. Positive reactions were determined by visual assessment, and a purple color reaction was recorded as positive.

3.2.5 Establishment of virus acquisition and test plants

Virus tested sweetpotato cv. Beauregard and Evangeline were graft inoculated with SPFMV-RC isolate 95-2 using scions from infected SOH plants. The isolate was maintained in SOH by serial mechanical inoculations and routinely tested for SPFMV by NCM-ELISA. Two wedge grafts were made per plant by inserting a single-node vine segment from the source plant into a slit in the stock plant. Only those on which scions survived for 3 weeks were used. Since titers in sweetpotato are often too low for detection by ELISA (Kokkinos et al., 2006; Clark et al., 2012), plants were assayed for successful inoculation by grafting onto the standard virus indicator plant, Brazilian morning glory (*Ipomoea setosa*), and only those that produced typical SPFMV symptoms were used for study. *Ipomoea hederacea* and *I. cordatotriloba* seedlings were mechanically inoculated with SPFMV-RC, isolate 95-2. Carborundum-dusted cotyledons of plants were rubbed approximately 5 to 7 days after planting with sap extracts from *I. nil* plants in which the isolate was maintained. Sap was obtained by grinding small leaf portions expressing symptoms in 1 ml of inoculation buffer (0.05 M sodium phosphate with 0.01 M diethyldithiocarbamic acid [DIECA]) using a sterilized mortar and pestle.

Plants were rinsed with distilled water after inoculation. Only those expressing typical SPFMV symptoms 3 weeks after inoculation were used for aphid transmission studies. For plants infected with the ‘natural’ mix of viruses, vine cuttings were obtained from B14, G-7 and established in 15 cm diameter clay pots. Scions from the naturally mixed infected plants were then used to graft inoculate 6 week old plants of *I. hederacea*, *I. cordatotriloba* and sweetpotato cv. Evangeline. In the case of cv. Evangeline, plants with scions that survived for 3 weeks were assayed on the indicator plant *I. setosa* to confirm successful SPFMV inoculation. Only plants that indexed as positive for SPFMV on *I. setosa* were used for aphid transmission studies. NCM-ELISA assays confirmed that mixed infected source plants had SPFMV and SPVG.

3.2.6 Transmission experiments with *Aphis gossypii* and *Myzus persicae*

SOH plants were used as test plants at the cotyledonary stage, approximately 5 to 7 days after sowing. The following treatments were carried out, each on 20 test plants per experiment that were repeated five times. Virus source plants were *I. hederacea*, *I. cordatotriloba*, and sweetpotato cvs. Beauregard and Evangeline for both singly infected with SPFMV-RC isolate 95-2 and the naturally mixed infected.

Adult apterae (non-winged) aphids of *M. persicae* and *A. gossypii* were removed using a camel’s hair brush from respective colonies, placed in separate Petri dishes lined with moist filter paper, and fasted for 2 hours. Fresh leaves expressing the most distinct symptoms of SPFMV, except for singly infected Beauregard and Evangeline on which all leaves were asymptomatic, were obtained from respective source plants 3 weeks after mechanical inoculation or 5-6 weeks after graft inoculation. Leaves corresponded to those that have high or mostly consistent titer (Wosula, E. N. unpublished). A single leaf

was placed on a moist filter paper under a dissecting light microscope. One adult aptera was transferred from the Petri dish using a camel's hair brush to the source leaf. Each aphid was monitored until it probed the leaf, as indicated by its assuming a resting position and then allowed an acquisition access period (AAP) of 3 seconds in preliminary tests and 30 seconds in subsequent tests after which it was transferred to an individual test plant. In each experiment, each source leaf was used for testing both *A. gossypii* and *M. persicae* and in most experiments, singly and mixed infected leaves were tested at the same time. Due to the number of transmissions involved, it was not possible to test all host species at the same time. The procedure was repeated for each of the eight acquisition source plants, 20 aphids per replicate, five replicates each (n = 100 for each acquisition source for each aphid). The individual source leaves used for acquisition were frozen in liquid nitrogen and then stored at -70°C for subsequent determination of virus titer using qRT-PCR. Individual viruliferous aphids were allowed an inoculation access period (IAP) of two hours, after which plants were sprayed with imidacloprid (Admire 2F, Bayer CropScience, Research Triangle Park, NC) at a rate of 0.9 g AI/l water to kill the aphids. Plants were left in the laboratory overnight, and then transferred back to a greenhouse for symptom monitoring. Plants were monitored daily for 3 weeks and those expressing typical SPFMV symptoms were recorded. Infected plants with transmissions from mixed infected sources were tested using NCM-ELISA to confirm presence of SPFMV.

Another transmission experiment was conducted with both aphid species using 3 seconds AAP on *I. hederacea* and *I. cordatotriloba* single infected, and Beauregard single and with mixed virus infection. The two aphid species were allowed to probe the

same individual leaf in each experiment but hosts and virus combinations were not necessarily tested at the same time due to time factor. Transmission efficiency of SPFMV was estimated based on by maximum likelihood using a Microsoft Excel Add-In PooledInfRate, Version 3.0 (Biggerstaff, 2006).

3.2.7 Quantitative reverse transcription (qRT-PCR) assays

The qRT-PCR assays were carried out according to procedures of Kokkinos and Clark (2006). Frozen leaf tissue (approximately 70 mg) was ground to a fine powder in liquid nitrogen using a mortar and pestle, and total RNA was extracted using Qiagen's RNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's directions. RNA concentrations were determined using a Nanodrop spectrophotometer (Nanodrop Technologies, Wilmington, DE). To eliminate residual DNA, total RNA samples were treated with DNase I (Invitrogen, Carlsbad CA). Quantitative RT-PCR assays were performed in 25 µl reaction volume mixtures containing 900 nM of each primer (forward and reverse), 200 nM of the MGB TaqMan probe, 12.5µl of 2X Master mix, 0.63µl RT enzyme mix (40X) which contains MultiScribe™ Reverse Transcriptase and RNase Inhibitor respectively, of the TaqMan One Step PCR Master Mix Reagents kit (Applied Biosystems, Foster City, CA), and 2.5 µl of template RNA. The same protocol was followed for the endogenous control reactions, which enable normalization of variation between sample extracts, except for the substitution of the target virus primer/probe set with 2.5 µl of the eukaryotic 18S rRNA primer/probe mix (VIC/ MGB Probe) (Applied Biosystems, Foster City, CA). The following qRT-PCR thermal cycling conditions were used: 48°C for 30 minutes (cDNA synthesis), 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at

60°C for 1 minute. All qRT-PCR reactions were performed on an ABI PRISM 7000 Sequence Detection System using MicroAmp optical 96-well reaction plates that are sealed with optical adhesive covers (Applied Biosystems). To compensate for any errors due to pipetting differences, duplicates of each sample were performed on each plate, and their threshold cycle (Ct) values were averaged during data analysis. A 5-fold standard curve of six dilutions was developed using a positive control of SPFMV-RC, isolate 95-2 RNA extract from SOH to test for any inhibition of optimal PCR conditions. In addition every plate contained duplicate wells with a no template control (NTC), a negative control with RNA extracts from healthy SOH and a positive control used for standard curves. Virus RNA titers (N) were normalized based on the mathematical formula $N = 2^{-\Delta Ct}$, where ΔCt is the difference between the threshold cycles (Ct) of the target virus and endogenous control (18S rRNA) obtained from their respective quantitative amplification plots, the Ct threshold was set by manually adjusting the base line and the threshold according to ABI PRISM 7000 Sequence Detection System manual instructions, to assure that it is in the linear phase of amplification for abundant 18S rRNA (Applied Biosystems, Foster City, CA). Data was log transformed and analyzed using Generalized Linear Model PROC GLM, PROC Means and PROC Corr procedures (SAS Software ver. 9.2. Cary, NC-USA).

3.3 Results

3.3.1 Transmission experiments with *Aphis gossypii* and *Myzus persicae*

The only successful transmission from sweetpotato was with *A. gossypii* from mixed infected Beauregard for both 3 seconds and 30 seconds acquisitions (Tables 3.1 and 3.2). SPFMV-RC transmission from mixed compared to singly infected sources

within host plants was significantly greater in *I. hederacea* with *A. gossypii*, but no significant differences were observed for other individual hosts (Table 3.1). Successful transmission of SPFMV did not occur with Evangeline as source, from either mixed or singly infected, or from singly infected Beauregard (Tables 3.1 and 3.2). The 3 seconds probe yielded successful transmissions of SPFMV in some host-virus combinations by both aphid species though at lower rates compared to the 30 seconds probes (Tables 3.1 and 3.2). According to the NCM-ELISA results, all test plants whose virus sources were mixed infected tested positive for SPFMV. All transmissions by *M. persicae* tested positive for SPFMV alone, while some transmissions by *A. gossypii* from all hosts tested positive for both SPFMV and SPVG, none tested positive for SPVG alone (Table 3.3). *Aphis gossypii* had a significantly higher transmission efficiency compared to *M. persicae* ($\chi^2 = 13.77$, $P = 0.0002$) when data were analyzed across all sources. When exposed simultaneously, *I. hederacea* was a better SPFMV source compared to other hosts ($\chi^2 = 20.66$, $P = <.0001$), and mixed infected leaves were better SPFMV sources than singly infected ones ($\chi^2 = 8.78$, $P = <.0030$).

Table 3.1. Sweet potato feathery mottle virus transmission from *Ipomoea* spp by *Aphis gossypii* and *Myzus persicae* following 30 seconds acquisition probes.

Acquisition source	% transmission			
	<i>A. gossypii</i>		<i>M. persicae</i>	
	Mean	CI	Mean	CI
<i>I. batatas</i> Beauregard SPFMV-RC	0	NT	0	NT
<i>I. batatas</i> Beauregard mixed	7	3.1 – 13.3	0	NT
<i>I. batatas</i> Evangeline SPFMV-RC	0	NT	0	NT
<i>I. batatas</i> Evangeline mixed	0	NT	0	NT
<i>I. cordatotriloba</i> SPFMV-RC	15	9.5 – 22.2	9	4.9 – 15.3
<i>I. cordatotriloba</i> mixed	20	13.1 – 28.7	8	3.8 – 14.6
<i>I. hederacea</i> SPFMV-RC	21	14.9 – 27.4	10	6.5 – 16.1
<i>I. hederacea</i> mixed	39	29.8 – 48.8	18	11.4 – 26.4
Control	0	NT	0	NT

Table 3.2. Sweet potato feathery mottle virus transmission from *Ipomoea* spp by *Aphis gossypii* and *Myzus persicae* following 3 seconds acquisition probes.

Acquisition source	% transmission			
	<i>A. gossypii</i>		<i>M. persicae</i>	
	Mean	CI	Mean	CI
<i>I. batatas</i> Beauregard SPFMV-RC	0	NT	0	NT
<i>I. batatas</i> Beauregard mixed	1	0.0 – 4.7	0	NT
<i>I. cordatotriloba</i> SPFMV-RC	11	6.7 - 19.5	3	0.8 -7.8
<i>I. hederacea</i> SPFMV-RC	10	6.0 – 18.3	10	6.0 – 18.3
Control	0	NT	0	NT

n = 100 for each acquisition source

Means within a column (acquisition sources) with same CI range are not significantly different (P>0.05).

(NT) No transmission occurred, therefore no confidence intervals can be calculated.

Table 3.3. Frequency of detection of Sweet potato feathery mottle virus (SPFMV), Sweet potato virus G (SPVG), and Sweet potato virus 2 (SPV2) in test plants following *Aphis gossypii* and *Myzus persicae* transmission from mixed infected hosts following a 30 seconds acquisition probe.

Host	<i>A. gossypii</i>			<i>M. persicae</i>		
	SPFMV	SPVG	SPFMV + SPVG	SPFMV	SPVG	SPFMV + SPVG
Beauregard	4	0	3	—	—	—
Evangeline	—	—	—	—	—	—
<i>I. cordatotriloba</i>	16	0	4	8	0	0
<i>I. hederacea</i>	36	0	3	18	0	0

n = 100 for each acquisition source

(-) Means not tested due to lack of successful transmission

3.3.2 SPFMV RNA titer in relation to aphid transmission

Quantification of SPFMV RNA titers in mixed and single infections revealed significant differences within some hosts. SPFMV titer was significantly greater in Beauregard mixed infected than in singly infected plants, but *I. cordatotriloba* mixed infected had a significantly lower titer than in singly infected. No significant differences between mixed and singly infected were observed within other hosts. Virus titers were generally significantly greater as was SPFMV transmission by *A. gossypii* and *M. persicae* in the *I. hederacea* and *I. cordatotriloba* singly infected morning glory plants than in the sweetpotato cvs. Beauregard and Evangeline (Fig. 3.1). Correlation analysis

revealed a significantly positive relationship between transmission and virus titer for both *A. gossypii* and *M. persicae* ($r = 0.74$, $P = 0.0131$; $r = 0.82$, $P = 0.0347$, respectively).

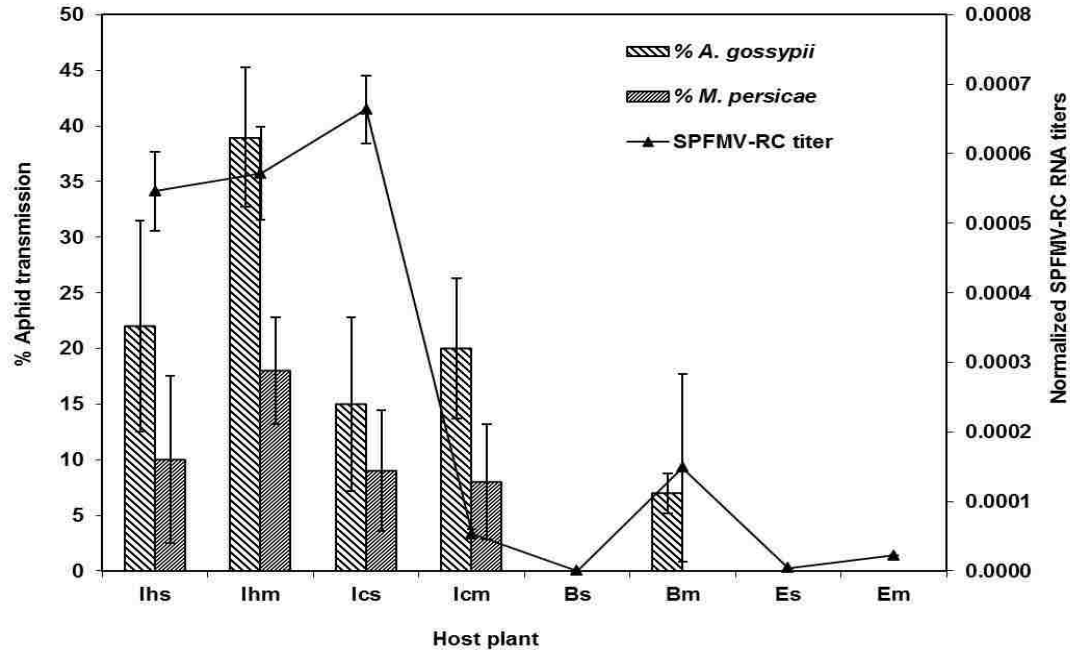


Fig. 3.1. Relative SPFMV-RC RNA titers and frequency of SPFMV transmission by *Aphis gossypii* and *Myzus persicae* in singly and mixed infected host plants (lh = *Ipomoea hederacea*, lc = *Ipomoea cordatotriloba*, B = sweetpotato cv. Beauregard, E = sweetpotato cv. Evangeline, s = singly infected, m = mixed infected).

3.4 Discussion

Dramatic differences in transmission of SPFMV occurred from different source plants depending on both host species and infection status. Based on the results under laboratory conditions, both *A. gossypii* and *M. persicae* are more likely to acquire and transmit SPFMV from infected *I. hederacea* and *I. cordatotriloba* than from infected sweetpotato cvs. Beauregard or Evangeline. Significantly greater SPFMV titers occurred in morning glory plants compared to sweetpotato plants. The results suggest that SPFMV replication in morning glory plants is more rapid compared to sweetpotato, hence high titers occur that enable easier acquisition and transmission by aphids. Kennedy and

Moyer (1982) revealed a similar trend in which transmission of SPFMV was lower when aphids were allowed to probe sweetpotato leaves compared with *I. setosa* leaves from graft inoculated plants. Other studies have also reported effect of host on virus accumulation e.g. weedy hairy nightshade *Solanum sarrachoides* common in potato growing regions is a better source of both *Potato virus Y* (PVY) and *Potato leafroll virus* (PLRV) (Alvarez and Srinivasan, 2005; Cervantes and Alvarez, 2011; Srinivasan and Alvarez, 2008). Differences among hosts in transmission rates by aphids could be attributed to varying levels of virus titer in source leaves depending on their position on the plant; a phenomenon previously reported involving transmission of PVY by *M. persicae* (De Bokx et al., 1978). There was a positive relationship between virus transmission and titer levels by both *A. gossypii* and *M. persicae* (Romanow et al., 1986).

SPFMV was transmitted at a greater rate across all host species, from mixed infected than singly infected plants but transmission rates from Evangeline and *I. cordatotriloba* with *M. persicae* did not differ. Virus titers did not differ between mixed and single infected plants within species except that titers were higher for mixed infected than singly infected Beauregard and singly infected than mixed infected *I. cordatotriloba*. The results suggest SPFMV titer accumulation in mixed infections may be enhanced as in the case of Beauregard, reduced as with *I. cordatotriloba*, or unaffected as with *I. hederacea*. However, despite the differences, mixed infections seem to be favorable virus sources compared to single infections indicating there could be other contributing factors apart from virus titer. Kokkinos and Clark (2006) reported significantly greater titers of SPFMV in sweetpotato plants when co-infected with a crinivirus *Sweet potato chlorotic stunt virus* (SPCSV) than in singly infected plants. However, there was no

significant difference in SPFMV titer in single and co-infections with two other sweetpotato potyviruses *Sweet potato virus G* (SPVG) and *Sweet potato virus 2* (SPV2) (Kokkinos and Clark, unpublished). The significant differences between mixed and singly infected virus titers in Beauregard therefore suggest there could be other unknown viruses contributing to enhanced titer in sweetpotato apart from the known SPVG and SPV2. Mixed virus infections in other systems are reported to cause severe symptoms and enhanced, reduced, or neutral effect on virus titers in either all or some of the viruses involved (Syller, 2012). Transmission of viruses by aphids from mixed infected plants differs, probably depending on titer enhancement or suppression by the given virus. For example, Hampton and Sylvester (1969) reported increased transmission of *Alfalfa mosaic virus* (AMV) by the pea aphid *Acyrtosiphon pisum* (Harris) when co-infected with *Pea streak virus* (PSV) compared to singly infected plants. However, they observed the reverse trend with PSV whose transmission efficiency was lower when co-infecting with AMV than when alone. The results are contrary to those of Pinto et al. (2008) who observed reduced transmission of *Zucchini yellow mosaic virus* (ZYMV), *Cucumber mosaic virus* (CMV), and *Papaya ring spot virus* (PRSV) from mixed infections compared to singly infected plants by both *A. gossypii* and *M. persicae*. Wintermantel et al. (2008) also reported reduced transmission of two criniviruses when co-infecting compared to single infections. Significant reduction of SPFMV titer in *I. cordatotriloba* mixed compared to single infected could be attributed to host effect on virus titer accumulation pattern. Some viruses may have enhanced or reduced titer in co-infections vs. single infections depending on host plant species or cultivar (Syller, 2012; Wintermantel et al., 2008).

Overall, *A. gossypii* had greater transmission efficiency than *M. persicae* but on some host-virus combinations, the transmission rate was very low and differences were not observed. *Aphis gossypii* was also able to transmit SPVG in some incidences from the mixed infected host plants, a trend that was not observed with *M. persicae* which only transmitted SPFMV. Although the results under laboratory conditions suggest that *A. gossypii* is a more efficient vector of SPFMV, field conditions present a different case, as the importance of a vector is determined based on its propensity, a measure determined by both vector efficiency and abundance (Irwin and Ruesink, 1986). Data on aphids caught in Louisiana sweetpotato fields using yellow sticky and pan traps indicate that *A. gossypii* is more abundant than *M. persicae* (Wosula et al., 2012). Single or multiple virus species or strains can be transmitted from mixed infected hosts depending on the aphid species (Rochow, 1972). Several publications have reported differences in virus transmission by various aphid species (Kennedy and Moyer, 1982; Souto et al., 2003; Verbeek et al., 2010). These differences in transmission are attributed to interaction of virus particles, helper component protein (HC-Pro), surface proteins on the aphid stylet, and virus coat protein that affect virus binding capacity and vector behavior (Ng and Falk, 2006; Syller, 2012). The transmission efficiency of SPFMV by *M. persicae* (0 to 18%) in our experiments was comparable to what has been reported for some potyviruses, for example PVY 4.7 to 71.1% (Cervantes and Alvarez, 2011; Davis et al., 2005; Ragsdale et al., 2001); *Soybean mosaic virus* (SMV) 18.6% , AMV 5 to 19%, and ZYMV 20 to 60% (Symmes and Perring, 2007). Transmission by *A. gossypii* was comparable to what has been reported on other potyviruses; for example ZYMV 27.5% (Yuan and Ullman, 1996). Successful transmission of SPFMV by *M. persicae* and *A.*

gossypii in as few as 3 seconds reveals how easily this virus can be acquired and spread in the fields by these aphid species. Earlier studies by McLean (1959) revealed a similar trend.

These findings demonstrate that transmission of SPFMV depends on host plant, aphid species, and infection status. The knowledge on transmission of SPFMV from *Ipomoea* hosts by the two aphid species *M. persicae* and *A. gossypii* is essential to accurately estimate transmission risks with regard to aphid species composition in sweetpotato fields. Further studies are needed to fully assess the role of morning glory species in epidemiology of sweetpotato potyviruses. Although SPFMV transmission from morning glory plants appeared greater than from sweetpotato, in the field sweetpotato is planted from vegetative cuttings many of which are already infected, whereas the morning glories germinate from true seed that are not initially infected. Field data indicate that the morning glories get infected during the months of June to July when SPFMV is already spreading among sweetpotato plants (Wosula et al., 2012). This suggests that the morning glory seedlings are not sources of primary inoculum. However, prevalence of *I. hederacea* and *I. cordatotriloba* in sweetpotato growing regions may serve as secondary sources of SPFMV, and during acute stage of infection when virus titers are highest, may play a role in SPFMV epidemiology. Differences in accumulation of SPFMV titers in mixed and single infected plants, depending on host, may play a role in adaptation and evolution of the virus and impact virus epidemiology. Regular trapping and identification of aphid species in sweetpotato fields could be essential in monitoring proportions of vector species and their impact on virus spread.

Recognition of the relative transmission efficiency of different vector species and suitability of different potential hosts for virus acquisition provides critical information for understanding the epidemiology and developing approaches to limit spread of SPFMV. In this study we found that *A. gossypii* is the most efficient vector of SPFMV, while in most crops *M. persicae* has been reported as the most efficient vector of non-persistent viruses (Verbeek et al., 2010). Field study results suggest that virus titers may vary dramatically under field conditions and may differ from greenhouse conditions (Wosula et al., 2012). The fact that SPFMV was acquired quickly and transmitted in a non-persistent manner also suggests that mineral/stylet oils, which have been reported to minimize virus spread of non-persistent aphid borne viruses (Simons and Zitter, 1980) should be evaluated for their potential to inhibit transmission of SPFMV, as another step towards devising integrated strategies to minimize virus spread. Further work is required to determine whether lack of transmission from sweetpotato cv. Evangeline is due to resistance to SPFMV or effect on the behavior of the vector. Preliminary studies reveal that *M. persicae* has a lower intrinsic rate of increase on Evangeline compared to Beauregard, and SPFMV titers are lower in Evangeline compared to Beauregard in field samples (Wosula, E. N. unpublished).

CHAPTER 4: THE ROLE OF APHID ABUNDANCE, SPECIES DIVERSITY AND VIRUS TITER IN THE SPREAD OF SWEETPOTATO POTYVIRUSES IN LOUISIANA²

4.1 Introduction

Sweetpotato [*Ipomoea batatas* (L.) Lam.], a member of the *Convolvulaceae* family, is cultivated widely in tropical and sub-tropical areas and ranks among the top 10 most important food crops worldwide (Loebenstein and Thottappilly, 2009). It is increasing in demand in the USA given its perception as a nutritious food with more processed products becoming available (Clark et al., 2010). In Africa and parts of Asia, sweetpotato is an important food security crop where it is relied upon during times of drought and famine when other crops fail (Gibson et al., 2009).

Sweetpotato is vegetatively propagated and is prone to accumulate viruses which cause cultivar yield decline and reduce storage root quality (Clark and Hoy, 2006; Clark et al., 2012). Of the sweetpotato viruses so far described, the most common worldwide is *Sweet potato feathery mottle virus* (SPFMV) (Clark et al., 2012; Loebenstein et al., 2009; Moyer and Salazar, 1989). In the USA, the most common viruses are the potyviruses: SPFMV, *Sweet potato virus G* (SPVG) and *Sweet potato virus 2* (SPV2, synonym = *Ipomoea vein mosaic virus*) (Clark et al., 2012; Moyer and Salazar, 1989; Souto et al., 2003). SPVG and SPV2 are known to occur in China, Africa, the USA, and were

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recently reported in Australia and the Pacific region (Rännäli et al., 2008; Tairo et al., 2006).

Effective implementation of disease management practices requires knowledge of numerous biological features that mediate pathogen transmission (Daugherty et al., 2009). In case of vector-borne pathogens, virus epidemiology could depend on vector abundance, vector species, host species, inoculum availability, pathogen strain and local climate (Daugherty et al., 2009; Davis et al., 2008). Although SPFMV, SPVG and SPV2 are prevalent in cultivated sweetpotato throughout the USA, and they frequently re-infect virus tested planting material, there is little knowledge about what factors affect their spread.

Sweetpotato potyviruses commonly occur as mixed infections in the field (Moyer and Salazar, 1989; Souto et al., 2003). Mixed infections of SPFMV, SPVG and SPV2 in sweetpotato cv. Beauregard usually show mild chlorotic spotting and veinal chlorosis or no symptoms, and cause yield losses of less than 15% (Kokkinos and Clark, 2006; Clark et al., 2010). Although mechanical transmission of sweetpotato potyviruses can occur under carefully controlled conditions, field transmission of these viruses is mainly by several aphid species (Loebenstein and Thottappilly, 2009; McLean, 1959; Stubbs and McLean, 1958) in a non-persistent manner (Kennedy et al., 1962; McLean, 1958). From past reports, aphids that transmit include *Aphis gossypii* Glover, *Aphis craccivora* Koch, *Myzus persicae* (Sulzer) and *Lipaphis pseudobrassicae* (Davis) (Loebenstein et al., 2009; McLean, 1959). Sweetpotato potyviruses can also infect other wild plants that are potential sources of inoculum (Clark et al., 2012; Loebenstein et al., 2009): mainly morning glory plants in the genus *Ipomoea* (Loebenstein et al., 2009). In Louisiana, the

morning glories *Ipomoea cordatotriloba* Dennst. (synonym *I. trichocarpa* Ell.) and *I. hederacea* Jacq. are common in and around sweetpotato fields, and *I. hederifolia* L., *I. lacunosa* L., and *I. wrightii* Wall. are also known wild hosts of SPFMV (Clark et al., 1986).

Aphids, particularly those in the family *Aphididae*, are of economic importance in temperate regions primarily due to their role as vectors of plant viruses, transmitting about 57% of known insect vectored plant viruses (Radcliffe and Ragsdale, 2002). They possess biological characteristics that make them effective in acquiring and transmitting plant viruses such as specialized morphs adapted to different functions, host plant alternation, exceptionally short life cycles due to parthenogenetic reproduction and a unique host finding behavior that involves sap sampling (Radcliffe and Ragsdale, 2002). The sap sampling behavior involves brief probes into the epidermal cells that may last just for a few seconds to determine acceptance or rejection of a plant for feeding (Powell et al., 2006). This behavior usually facilitates transmission of non-persistent viruses whose titers are usually higher in epidermal and sub-epidermal plant cells (Radcliffe and Ragsdale, 2002). Aphids may take as little as 3 to 35 seconds to acquire and inoculate non-persistent viruses, but transmissibility of these viruses is lost within a period of 1 to 2 hours (Stubbs and McLean, 1958; Wosula et al., 2012). Therefore, the progress of virus spread in the field depends on the number of vectors alighting and probing plants (vector activity) as well as the natural ability of each aphid species to inoculate the virus (vector propensity) (Davis et al., 2008). Effective transmission of these viruses can easily be carried out by transient aphid species that do not necessarily colonize the host plant (Davis et al., 2008; Racciah et al., 1985; Radcliffe and Ragsdale, 2002).

Aphid flight activity and species diversity have been monitored in other crop systems with the aim of understanding virus spread, and timing of control strategies such as applications of insecticides, crop oils or defoliant (Radcliffe and Ragsdale, 2002). In sweetpotato, the only recent study of aphid activity in relation to spread of sweet potato virus disease (SPVD) found that aphids were present throughout the study period in Uganda (Byamukama et al., 2004). SPVD is caused by synergism between the aphid transmitted SPFMV and whitefly transmitted *Sweet potato chlorotic stunt virus* (SPCSV). The authors did not report on aphid species diversity, but emphasized more the relationship between whiteflies and spread of SPCSV rather than aphid spread of SPFMV. In Louisiana during the 1950s, Kantack et al (1960) trapped aphids using tangle foot traps in sweetpotato fields in south Louisiana. The authors captured several aphid species, with the majority being *A. gossypii*, *M. persicae* and *Macrosiphum euphorbiae*. Yellow and green pan traps, and yellow sticky traps have been successfully used to monitor aphid flight activity and capture for species identification (Davis et al., 2008; DiFonzo et al., 1997; Radcliffe and Ragsdale, 2002). Although most studies report a positive relationship between aphid abundance and virus spread, there are also cases where no relationship was found depending on the season (Thomas et al., 1997). Several studies have also reported fluctuation in virus spread incidences depending on prevailing aphid species diversity in terms of number of vector species and population density of individual species (DiFonzo et al., 1997; Radcliffe and Ragsdale, 2002; Davis et al., 2008).

Aphid captures provide only an indirect measure of risk of virus spread. Therefore, other strategies such as exposure of sentinel plants at varying intervals at study

sites, or trapping live aphids and directly assaying for their ability to acquire and transmit viruses are commonly used to relate aphid flight activity to virus spread (Radcliffe and Ragsdale, 2002). Virus titer in host plants has been reported to vary depending on host species, infection status, growth stage, and season and this may impact virus acquisition and transmission by vectors (Banik and Zitter, 1990; De Bokx et al., 1978; Dovas et al., 2002; Kokkinos and Clark, 2006; Rochow, 1972; Torrance and Dolby, 1984; Wosula et al., 2012). Higher virus titers in source leaves are related with increased transmission rates by aphids (Banik and Zitter, 1990; De Bokx et al., 1978; Wosula et al., 2012), indicating titer levels may play an important role in spread of viruses apart from presence of vectors.

The aim of this study was to monitor aphid abundance, aphid species diversity and field virus titers in relation to the spread of potyviruses in Louisiana sweetpotato fields (plant beds and production fields). This knowledge is essential in designing proper management strategies to obtain the healthiest possible planting materials and minimize the impact of these potyviruses on sweetpotato yield and root quality.

4.2 Materials and methods

4.2.1 Aphid trapping

Aphids were trapped using yellow sticky traps for total population counts for three consecutive years (2009 to 2011), and pan traps for use in species identification for four consecutive years (2008 to 2011) during the months of March/April to September at six sites in Louisiana. Two of the sites in Louisiana, the Burden Research Center (BRC) in East Baton Rouge parish and the Sweet Potato Research Station (SPRS) at Chase in Franklin parish are research stations, and the other four were commercial sweetpotato

farms located in St Landry, West Carroll, Morehouse, and Franklin parishes of Louisiana. Three sites (BRC, SPRS and St Landry) were sampled during the entire study period, while the other three sites all located in north Louisiana were each sampled for one year.

Yellow sticky traps (7.35 x 12.25 cm) with double-sided adhesive (Whitemire Micro-Gen Research Laboratories Inc., St Louis, MO) were attached to dark green stakes (120 cm height x 0.5 cm diameter, Woodstream Corporation, Lititz, PA) using two small binder clips (Staples Inc. Framingham, MA) at 90 cm above the ground. The yellow sticky traps were exposed for weekly intervals at five different positions within each field, four at the corners and one at the center. Three sets of yellow and green pan traps were also exposed for weekly intervals in each field. One set was placed at the center, while the other two were placed at two diagonally opposed corners. The pan traps were small, rectangular, 1.4-liter plastic dishes (Servin Saver, Rubbermaid, Wooster, OH) containing yellow and green tiles 7.35 x 7.35 cm (Imola – Cooperativa Ceramica D' Imola S.C., Vittorio Veneto, Italia) and 50 ml of a 50:50 volume mixture of propylene glycol and water. They were supported in tomato wire cages just above the sweetpotato canopy. Sticky traps were wrapped in clear wrap films to prevent them from adhering together when collected from the field. Aphids on sticky traps were counted with the aid of a dissecting microscope (Bausch and Lomb, Rochester, NY) using 7x magnification. Pan trap contents were brought to the laboratory and insects extracted using a 7.5 cm-diameter Büchner funnel lined with filter paper, and inserted in a conical flask with a rubber pipe connected to a suction pump. Aphids collected from pan traps were preserved in 1.5 ml vials containing 95% ethanol for later identification. Aphids were identified to species or genus level with aid of taxonomic keys based on assessment

characteristics including wing, abdomen, siphunculi, cauda, head and antenna morphology using magnification of 35x (dissecting microscope) or 160x (compound microscope) (Blackman and Eastop, 2006; Pike et al., 2003; Smith et al., 1992; Voegtlin et al., 2004).

4.2.2 Sentinel plants

Ipomoea setosa (Brazilian morning glory) seedlings at the cotyledonary stage were used to monitor the spread of potyviruses. Plants were grown from seed (4/pot) in the greenhouse under wide temperature (10-32°C) and humidity (21-98%) ranges, in 15-cm-diameter plastic pots (Belden Plastics, St Paul, MN.) containing autoclaved soil mix consisting of 1 part river silt, 1 part sand, 1 part Jiffy-Mix Plus (Jiffy Products of America Inc., Norwalk, OH) and 3.5 g/pot Osmocote 14-14-14 (Scotts-Sierra Horticultural Products Co., Marysville, OH). Plants were not sprayed with insecticides. Single pots with up to 2 to 4 seedlings, depending on germination, were placed in the field next to sticky traps for a total of five pots in each field. Soil moisture was maintained by placing pots in water filled non perforated plastic seedling trays (Belden Plastics, St Paul, MN). The plants were removed from the field on a weekly basis and placed in Bug Dorm cages (MegaView Science Co. Ltd, Taichung, Taiwan) in the greenhouse. They were monitored for expression of virus symptoms for an additional two weeks. Plants showing symptoms were recorded and leaves sampled for testing of SPFMV, SPVG and SPV2 using NCM-ELISA.

4.2.3 NCM-ELISA assays

The most widely used serological method (Clark et al., 2010) for detection of sweetpotato viruses is a membrane immuno-binding assay known as nitrocellulose membrane enzyme linked immunosorbent assay (NCM-ELISA). Since virus titers are often very low in sweetpotato, NCM-ELISA is generally used after grafting to indicator hosts such as *I. setosa*. Leaf tissue was collected from symptomatic sentinel plants and assayed using antisera produced to the russet crack strain of SPFMV provided by J.W. Moyer (North Carolina State University, Raleigh), or antisera to isolates from Louisiana of SPVG and SPV2 provided by S. Fuentes (International Potato Center, Lima, Peru). A small piece (~ 1 cm²) was collected from three different leaves with symptoms from each Brazilian morning glory plant, combined, placed in a “Universal” extraction bag (Article No. 430100; BIOREBA, Reinach BL 1, Switzerland), and homogenized using a HOMEX6 homogenizer (BIOREBA) in 8 ml of Tris-buffered saline (TBS = 0.02 M Tris base, 0.50 M NaCl) pH 7.5 containing 0.2% of sodium sulfite (Na₂SO₃). Two ml of the extracted tissue were transferred to a 2 ml microfuge tube and allowed to stand for 30 to 45 minutes at room temperature. The clarified sap extract (50 µl) was blotted onto a TBS buffer-saturated nitrocellulose membrane and air-dried for 20 to 30 minutes. All the incubations and washings were done at room temperature in a shaker with gentle agitation (50 rpm for incubations and 100 rpm for washings). The membranes were blocked in TBS containing 2% powdered milk and 2% Triton X-100 (Sigma-Aldrich, St. Louis, MO) for 1 hour. The blocking solution was discarded and the membranes rinsed with T-TBS [TBS containing 0.05% Tween 20 (Sigma-Aldrich)]. The first antibody (polyclonal specific to SPFMV, SPVG or SPV2), was cross absorbed in healthy Brazilian

morning glory extract (1 g tissue homogenized in 25 mL of TBS containing $2 \text{ g}\cdot\text{L}^{-1}$ sodium sulfite, $20 \text{ g}\cdot\text{L}^{-1}$ Carnation skim milk (Nestlé USA, Inc., Solon, OH), and $0.2 \text{ g}\cdot\text{L}^{-1}$ sodium azide – pH 7.5) for 45 minutes at $37 \text{ }^\circ\text{C}$ and was then added to the membranes and incubated for 1 hour. The membranes were washed in T-TBS four times for 3 minutes each. The second antibody (goat anti-rabbit) conjugated with alkaline phosphatase (Jackson ImmunoResearch Laboratories Inc. West Grove, PA) diluted in TBS containing 2% powdered milk, was added to the membranes and incubated for 1 hour. The membranes were washed as before and the presence of bound antibody was visualized by the addition of substrate solution [10 mg of nitrobenzene tetrazolium in 100 μL dimethyl formamide followed by 5 mg of 5-bromo-3-chloro indolyl phosphate in 100 μL dimethyl formamide added to 30 mL substrate buffer (0.1 M Tris HCl, 0.1 M NaCl, 5 mM MgCl_2 , pH 9.5)]. The color reaction was stopped after 30 minutes by washing with deionized water. Positive reactions were determined by visual assessment, and a purple color reaction was recorded as positive.

4.2.4 Virus titer quantification

Quantitative reverse transcription PCR (qRT-PCR) assays are the most reliable assays for relative quantification of viruses from sweetpotato (Clark et al., 2012; Kokkinos and Clark, 2006). These assays were used for virus titer quantification from sweetpotato plants cv. Beauregard in plant beds and production fields at BRC on a weekly basis from May to September during 2010 and 2011. Leaf portions ($\sim 9 \text{ cm}^2$) were collected arbitrarily from lower vine sections (preliminary experiments revealed greater SPFMV, SPVG and SPV2 titers in lower leaves than middle or upper leaves of sweetpotato plants under field conditions) of five different plants and combined into a

single composite sample in small envelopes. Five samples were collected each week, leaves were immediately placed in liquid nitrogen and transferred to the laboratory where they were stored under refrigeration at -70°C until extraction of RNA.

Assays were carried out according to procedures of Kokkinos and Clark (2006). Frozen leaf tissue (approximately 70 mg) was ground to a fine powder in liquid nitrogen using a mortar and pestle, and total RNA was extracted using Qiagen's RNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's directions. RNA concentrations were determined based on absorption at 260 and 280 nm using a Nanodrop spectrophotometer (Nanodrop Technologies, Wilmington, DE). To eliminate residual DNA, total RNA samples were treated with DNase I (Invitrogen, Carlsbad CA) with RNA concentrations adjusted according to instructions based on amount in each sample ($\text{ng}/\mu\text{l}$) according to spectrophotometer readings. Quantitative RT-PCR assays were performed in 25 μl reaction volume mixtures containing 900 nM of each primer (forward and reverse), 200 nM of the MGB TaqMan probe, 12.5 μl of 2X Master mix, 0.63 μl RT enzyme mix (40X) which contains MultiScribe™ Reverse Transcriptase and RNase Inhibitor respectively, of the TaqMan One Step PCR Master Mix Reagents kit (Applied Biosystems, Foster City, CA), and 2.5 μl of template RNA. The same protocol was followed for the endogenous control reactions, which enable normalization of variation between sample extracts, except that 2.5 μl of the eukaryotic 18S rRNA primer/probe mix (VIC/ MGB Probe) (Applied Biosystems Foster City, CA) was substituted for the target virus primer/probe set. The following q RT-PCR thermal cycling conditions were used: 48°C for 30 min (cDNA synthesis), 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at

60°C for 1 minute. Quantitative RT-PCR reactions were performed on an ABI PRISM 7000 Sequence Detection System using MicroAmp optical 96-well reaction plates sealed with optical adhesive covers (Applied Biosystems). To compensate for any errors due to pipetting differences, duplicates of each sample were performed on each plate, and their threshold cycle (Ct) values were averaged during data analysis. A 5-fold standard curve of six dilutions was developed using a positive control of SPFMV-RC, isolate 95-2, SPVG and SPV2 RNA extracts from *Ipomoea nil* cultivar Scarlet O'Hara (SOH) to test for any inhibition of optimal PCR conditions. In addition every plate contained duplicate wells with a no template control (NTC), a negative control with RNA extracts from healthy SOH and a positive control used for standard curves. Virus RNA titers (N) were normalized based on the mathematical formula $N = 2^{-\Delta Ct}$, where ΔCt is the difference between the threshold cycles (Ct) of the target virus and endogenous control (18S rRNA) obtained from their respective quantitative amplification plots, the Ct threshold was set by manually adjusting the base line and the threshold according to ABI PRISM 7000 Sequence Detection System manual instructions (Applied Biosystems, Foster City, CA).

4.2.5 Morning glory weeds

Study sites in Louisiana were monitored for morning glory plants growing as weeds; their species diversity and time of first virus symptom appearance were recorded. Plant species were identified based on an identification key by Rogers and Oliver (1982).

4.2.6 Virus vector testing

Two aphid species that had not previously been tested as vectors of sweetpotato potyviruses, *Rhopalosiphum maidis* Fitch and *Rhopalosiphum Padi* (L.) were tested for their ability to transmit SPFMV. These two aphid species were selected because they

were identified among those captured in pan traps, and it was possible to establish their colonies. *Rhopalosiphum padi* was collected from winter wheat at the LSU AgCenter Sweet Potato Research Station (Chase) in Franklin parish, Louisiana in February 2012. *Rhopalosiphum maidis* was collected from winter wheat at the LSU AgCenter Macon Ridge Research Station (Franklin parish), Louisiana in March 2011. Aphid colonies were established on wheat plants (*Triticum aestivum*) cv. LA 841 (not a host for sweetpotato viruses) from a group of apterae and maintained under laboratory conditions in screened cages at room temperature (20-22°C) and a 16L: 8D photoperiod. Plants were grown in a growth chamber with temperature set at 25°C, 14L: 10D photoperiod and humidity at 80%. A cohort of 5 to 10 aphids was placed on fresh plants using a paint brush to establish new colonies every 2 to 3 weeks. In addition, *M. persicae* maintained according to Wosula et al. (2012) was used as a comparative standard since it is a known efficient vector of SPFMV.

Ipomoea cordatotriloba was used as the source plant of SPFMV because our previous study indicated *M. persicae* and *A. gossypii* transmit SPFMV more readily from this host than sweetpotato. SPFMV was established and maintained in *Ipomoea nil* cv. Scarlet O'Hara (SOH) as described in Wosula et al. (2012) from which it was mechanically inoculated to *I. cordatotriloba*. SOH plants were used as test plants at the cotyledonary stage, approximately 5 to 7 days after sowing. The transmission experiment treatments, replicates and procedures were carried out as described in Wosula et al. (2012) except in this experiment aphids were allowed to probe for 1 minute instead of 30 seconds.

4.2.7 Data analysis

Means and their respective standard errors for aphid counts on yellow sticky traps, aphid species counts in pan traps, percent infected sentinel plants and virus titer were generated using SAS PROC MEANS procedures. Data for comparison of aphid numbers on yellow sticky traps at different locations within the field were log transformed and subjected to SAS PROC Generalized Linear Model (GLM) procedures. PROC CORR procedure with Pearson's correlation coefficient was used to test for the relationship between aphid number and infection of sentinel plants. Transmission efficiency was estimated as number of infected test plants divided by total number of test plants, expressed as percentage. Maximum likelihood ratios were generated, and confidence intervals calculated using Microsoft Excel add-in *PooledInfRate* Version 3.0 (Biggerstaff, 2006).

4.3 Results

4.3.1 Aphid trapping

Although populations fluctuated with time, aphids were captured on yellow sticky traps throughout the monitoring period from March/April to September for the 3 year period at six sites in Louisiana (Figs. 4.1, 4.2 and 4.3). At BRC, the major aphid peaks occurred early in the growing season (April to May), and late in the growing season (August to September), while peaks were very rare or absent during the period of June to July (Fig. 4.1). Aphid peaks were erratic during 2009 and 2011 at SPRS, but in 2010 three distinct peaks were observed in May, July and September (Fig. 4.1). St Landry parish recorded major aphid peaks early in the season (April to May) during the three year period, and very low populations the rest of the growing season (June to September)

(Fig. 4.2). West Carroll parish recorded low aphid populations most of the period except for a minor peak in July, and a major peak in September that was probably occasioned by aphids migrating from a corn field that was harvested at that time (Fig. 4.2). Morehouse parish had erratic aphid peaks but major ones occurred during the period of July to September (Fig. 4.3). Franklin parish displayed three distinct aphid peaks during the period of May, July and late August (Fig. 4.3). Additional analysis of log transformed aphid count data revealed no significant differences in aphid captures on sticky traps based on trap location within the field across all sites during the study period (F value = 2.04; $P = 0.0863$).

Total aphids captured in yellow and green pan traps for the four-year period comprised of 26 species (Tables 4.1). Aphid species were diverse and with variable percentage compositions during the four-year study period. In 2008, five aphid species: *A. gossypii*, *R. padi*, *Forda formicaria* (von Heyden), *R. maidis* and *T. trifolii* (Monell) comprised 93% of total aphids captured, with two, *A. gossypii* and *R. padi*, comprising 75%. In 2009, six aphid species: *A. gossypii*, *F. formicaria*, *Macrosiphum euphorbiae*, *R. padi* and *Sipha flava* (Forbes) accounted for 92% of total aphids captured, with *A. gossypii* and *R. padi* comprising 74%. In 2010 six aphid species: *A. gossypii*, *Lipaphis pseudobrassicae*, *M. persicae*, *R. padi* and *T. trifolii* accounted for 94% of total aphids captured, and *A. gossypii* and *R. padi* comprised 51%. In 2011 seven aphid species: *A. gossypii*, *F. formicaria*, *L. pseudobrassicae*, *Uroleucon ambrosiae* (Thomas), *M. persicae*, *R. padi*, and *T. trifolii* accounted for 83% of total aphids captured, and *A. gossypii* and *R. padi* comprised 47%. The known efficient vectors of sweetpotato potyviruses, *M. persicae* and *A. gossypii*, when combined accounted for 56%, 40%, 26%

and 18% in 2008, 2009, 2010 and 2011, respectively. The dominant species at most of the locations were *A. gossypii* and *R. padi*, other two important species were *M. persicae* and *T. trifolii*. These four species were present at Burden Research Center with major peaks early in the season (April to May), but only *A. gossypii* and *R. padi* were present during the period of June to September (Fig. 4.4). Sweet Potato Research Station recorded low captures of *A. gossypii* and *R. padi* early in the season, but high populations occurred with major peaks during the period of mid-June to late July. *Therioaphis trifolii* occurred only early in the season, while *M. persicae* was mostly absent (Fig. 4.4). These four species were present at St Landry parish with major peaks occurring early in the season, but during the period of June to September only *A. gossypii* and *R. padi* were captured in low numbers (Fig. 4.5). West Carroll parish recorded mostly *A. gossypii* and *R. padi* with major peaks in mid-June to late July, while *M. persicae* and *T. trifolii* were rarely captured (Fig. 4.5). Morehouse parish had erratic peaks with *R. padi* and *T. trifolii* occurring early in the season, while *A. gossypii* and *R. padi* occurred mainly in July to September (Fig 4.6). Franklin parish had *R. padi* and *T. trifolii* early in the season (April to May), while *A. gossypii* and *R. padi* were mostly present during the period of mid-June to mid-August (Fig 4.6). Total aphids captured in pan traps at all sites during the entire study period were significantly higher in yellow pan traps (1679) than green pan traps (920) ($\chi^2 = 5.4$, $P = 0.0196$). Among the most prevalent aphid species (having at least 10% of total population in any of the years), *R. padi* was the only species that had a significant difference in captures in different color pan traps with more caught in yellow traps ($\chi^2 = 3.85$, $P = 0.0495$).

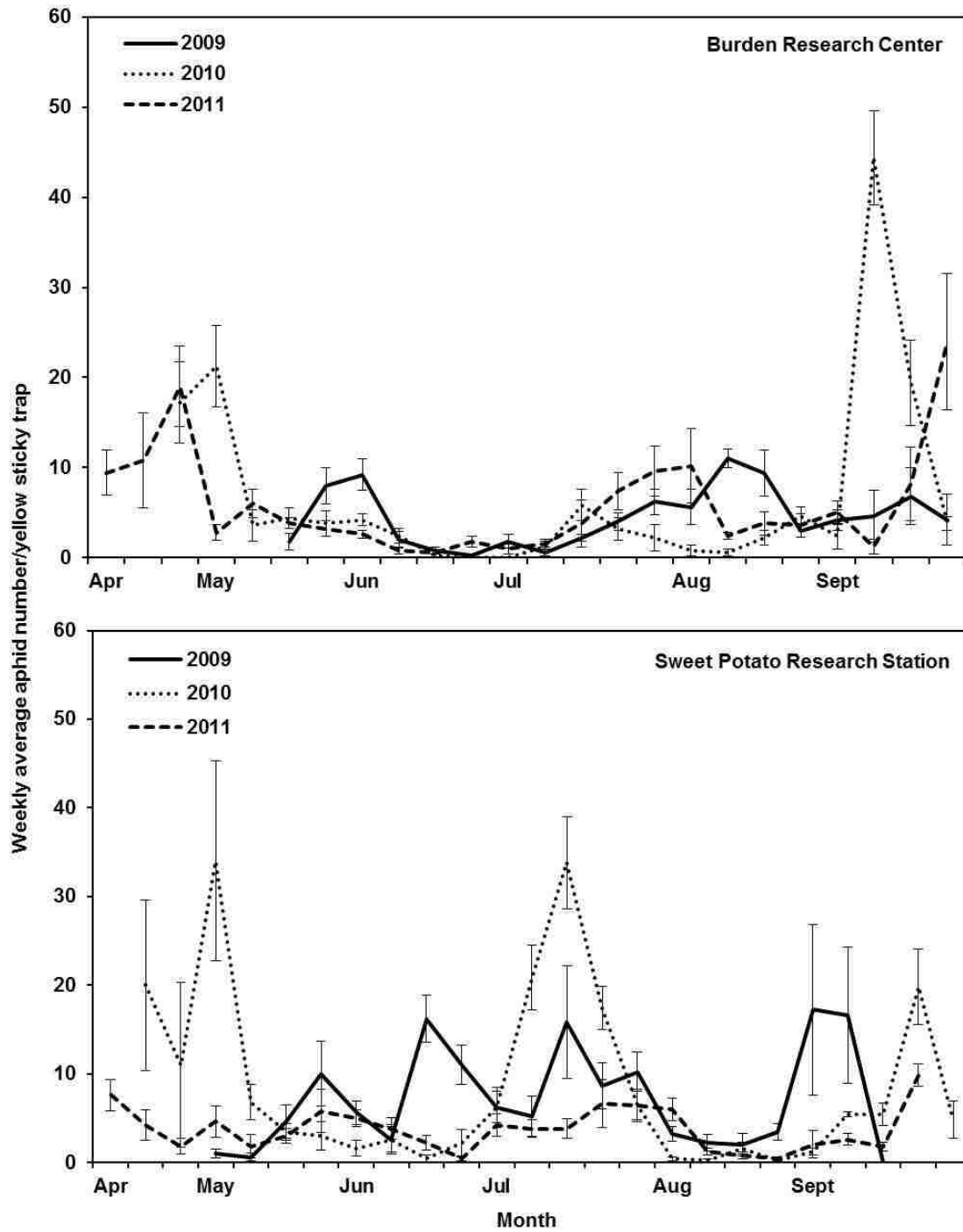


Fig 4.1. Weekly average number of aphids captured on yellow sticky traps (number/trap) in sweetpotato fields at Burden Research Center and Sweet Potato Research Station 2009 to 2011 (Means \pm SE).

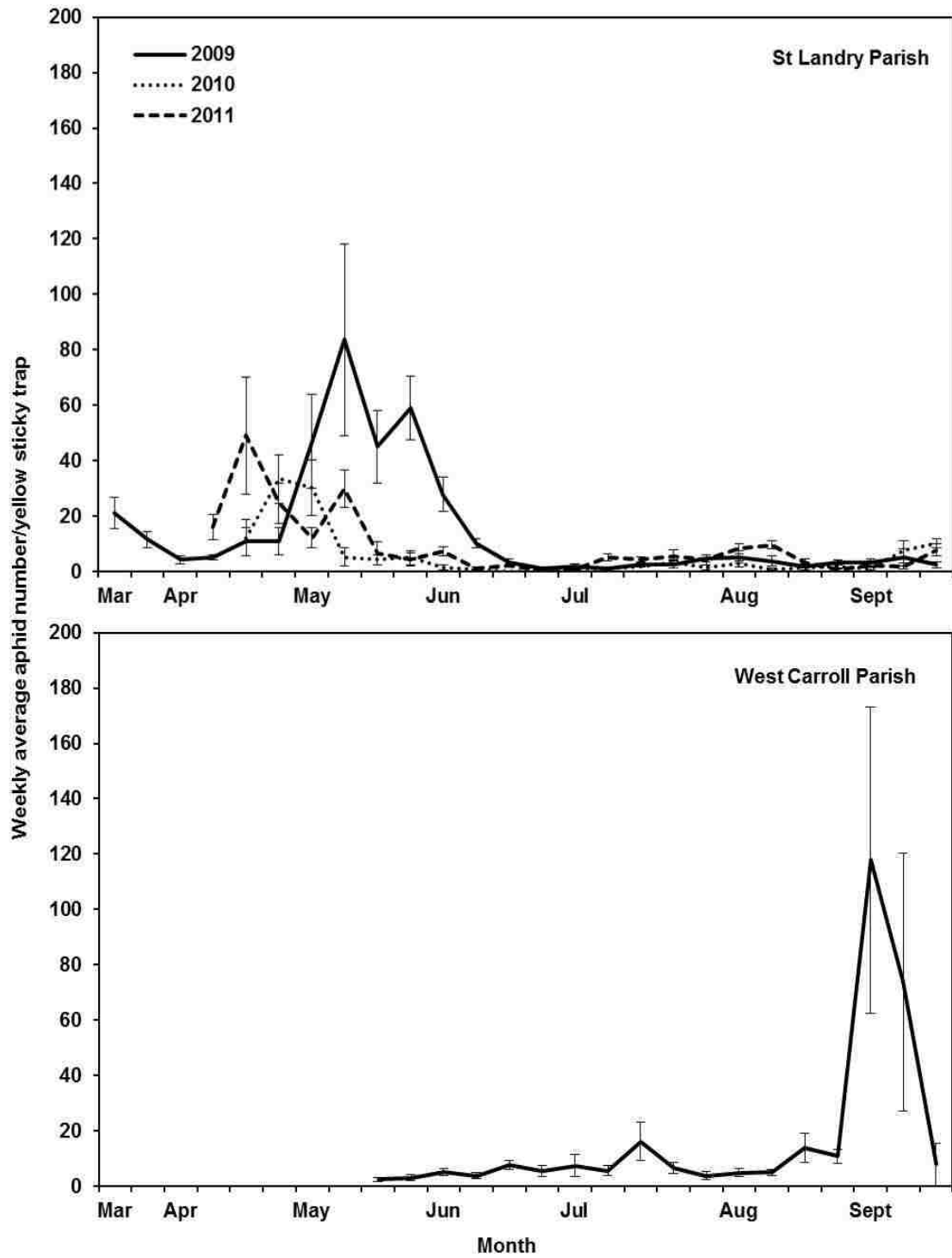


Fig. 4.2. Weekly average number of aphids captured on yellow sticky traps (number/trap) in sweetpotato fields at St Landry parish (2009 to 2011) and West Carroll parish (2009) (Means \pm SE).

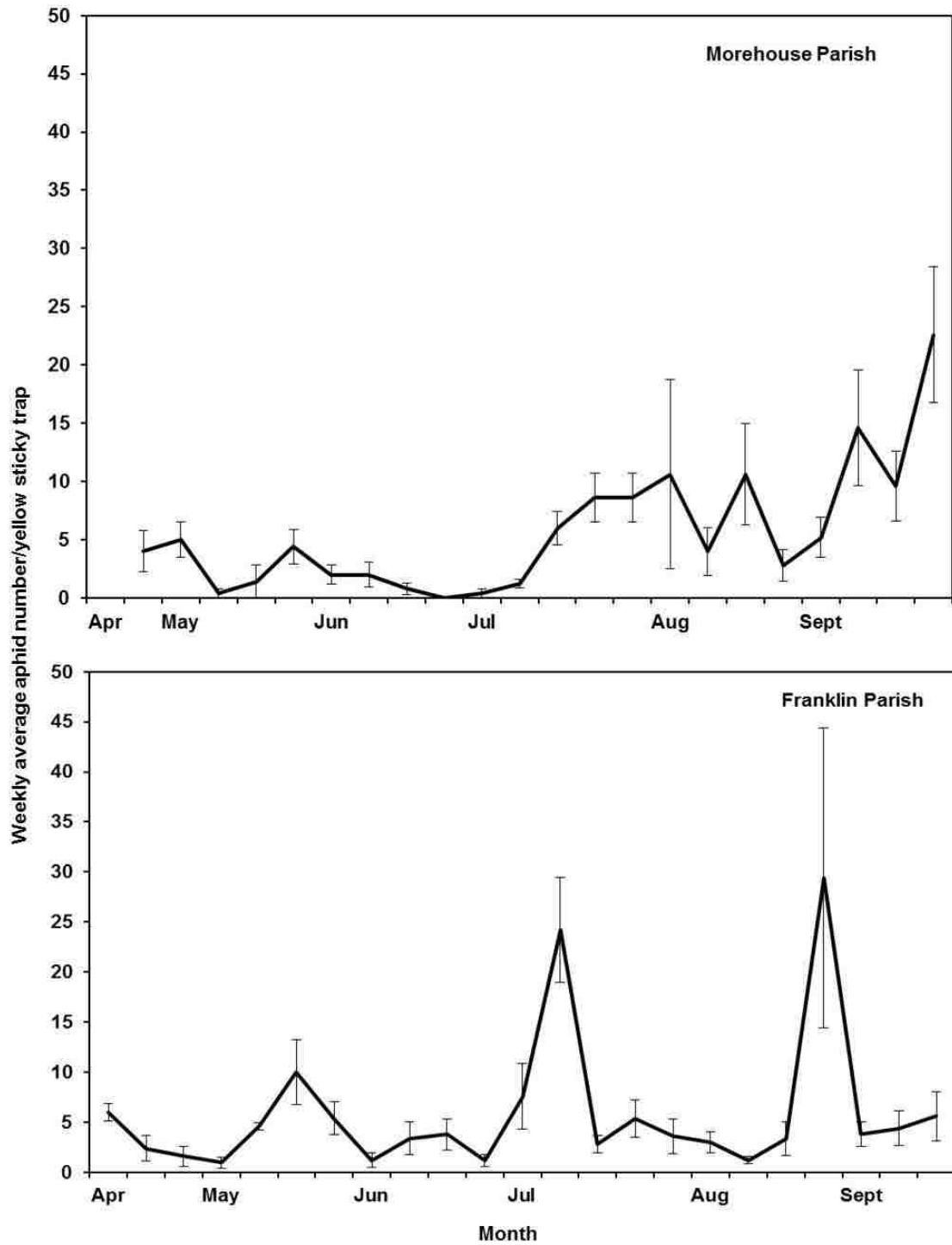


Fig. 4.3. Weekly average number of aphids captured on yellow sticky traps (number/trap) in sweetpotato fields at Morehouse parish (2010) and Franklin parish (2011) (Means \pm SE).

4.3.2 Sentinel plants

Although sentinel plants were placed in the fields throughout the study period, a majority of the infections occurred during the months of June to August with the highest infections in July. Seventy eight percent of the total infections of sentinel plants at all locations occurred during July and August. The experimental plots at Burden Research Center recorded infections during the period of late June to mid-August for all the three years (Fig. 4.7). The Sweet Potato Research Station had erratic infections occurring during the period of early July to September (Fig 4.7). St Landry parish had few infections in the months of April and May, but most infections occurred during the period of late June to September (Fig 4.8). The highest infection rate of sentinel plants occurred at the three commercial farms, West Carroll (2009), Morehouse (2010) and Franklin (2011). Most infections occurred during the period of June to mid-August except for Morehouse which had another infection peak in September (Fig 4.8). The highest percent total infection of sentinel plants at all sites combined was 5% in 2009 and 2010, while 2011 had the least present infection (4%). Symptomatic plants tested positive for SPFMV (97 to 100%), SPVG (9 to 15%) and SPV2 (1 to 6%) (Table 4.2). Correlation analysis revealed a positive significant relationship between the total number of aphids captured on sticky traps and infection of sentinel plants at Morehouse parish ($r = 0.45$; $P = 0.0358$), but Burden Research Center had a significant negative relationship ($r = -0.44$; $P = 0.0290$). The rest of the locations had no significant relationships; Sweet Potato Research Center ($r = 0.35$; $P = 0.0882$), St Landry parish ($r = -0.31$; $P = 0.1178$), West Carroll parish ($r = -0.13$; $P = 0.6131$), Franklin parish ($r = 0.11$; $P = 0.6039$). Correlation

analysis with captures of the known efficient vector *A. gossypii* and infection of sentinel plants showed non-significant relationships at all locations.

Table 4.1. Total number and percentage composition of aphids captured in green and yellow pan traps in sweetpotato fields at sites in Louisiana, 2008 to 2011.

Aphid species	2008 n (%)	2009 n (%)	2010 n (%)	2011 n (%)
<i>Acyrtosiphum pisum</i> (Harris)	0 (0.0)	0 (0.0)	0 (0.0)	4 (0.8)
<i>Amphorophora rubi</i> (Kalt.)	0 (0.0)	1 (0.1)	4 (0.5)	1 (0.2)
<i>Anoecia</i> sp	0 (0.0)	1 (0.1)	0 (0.0)	3 (0.6)
<i>Aphis craccivora</i> Koch	0 (0.0)	0 (0.0)	0 (0.0)	25 (5.2)
<i>Aphis gossypii</i> Glover	117 (56.3)	235 (36.8)	138 (17.7)	68 (14.3)
<i>Aphis nasturtii</i> (Kalt.)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)
<i>Aphis</i> sp.	0 (0.0)	2 (0.2)	1 (0.1)	6 (1.2)
<i>Chaitophorus populicola</i> (Thomas)	0 (0.0)	1 (0.1)	3 (0.4)	0 (0.0)
<i>Colopha</i> sp.	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)
<i>Drepanaphis</i> sp.	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)
<i>Drepanosiphum</i> sp.	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)
<i>Eriosoma</i> sp.	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.4)
<i>Forda formicaria</i> (von Heyden)	16 (7.7)	32 (4.9)	25 (3.2)	55 (11.4)
<i>Hylopterus</i> sp.	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.6)
<i>Lipaphis pseudobrassicae</i> (Davis)	4 (1.9)	20 (3.1)	53 (6.8)	12 (2.5)
<i>Macrosiphum euphorbiae</i> (Thomas)	0 (0.0)	27 (4.2)	4 (0.5)	11 (2.3)
<i>Myzus persicae</i> (Sulzer)	0 (0.0)	18 (2.8)	121 (15.3)	27 (5.5)
<i>Rhopalosiphum maidis</i> (Fitch)	15 (7.2)	0 (0.0)	0 (0.0)	7 (1.2)
<i>Rhopalosiphum padi</i> (L.)	39 (18.8)	238 (37.4)	262 (33.3)	157 (33.0)
<i>Rhopalomyzus</i> sp.	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)
<i>Sarucallis kahawaluokalani</i> (Kirkaldy)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)
<i>Schizaphis graminum</i> (Rondani)	0 (0.0)	0 (0.0)	0 (0.0)	4 (0.8)
<i>Sipha flava</i> (Forbes)	2 (0.9)	23 (3.6)	9 (1.1)	0 (0.0)
<i>Therioaphis trifolii</i> (Monell)	8 (3.4)	35 (5.5)	99 (12.6)	59 (12.2)
<i>Uroleucon ambrosiae</i> (Thomas)	2 (1.0)	4 (0.5)	65 (8.4)	19 (4.0)
<i>Utamphorophora</i> sp.	2 (0.9)	0 (0.0)	0 (0.0)	0.0
Unidentified/damaged	1 (0.5)	9 (1.4)	1 (0.1)	8 (1.6)
Aphids caught in yellow pan traps	138 (66.3)	459 (72.2)	482 (61.4)	303 (63.8)
Aphids caught in green pan traps	70 (33.7)	177 (27.8)	303 (38.6)	172 (36.2)
Total no. of aphids caught	208	636	785	475

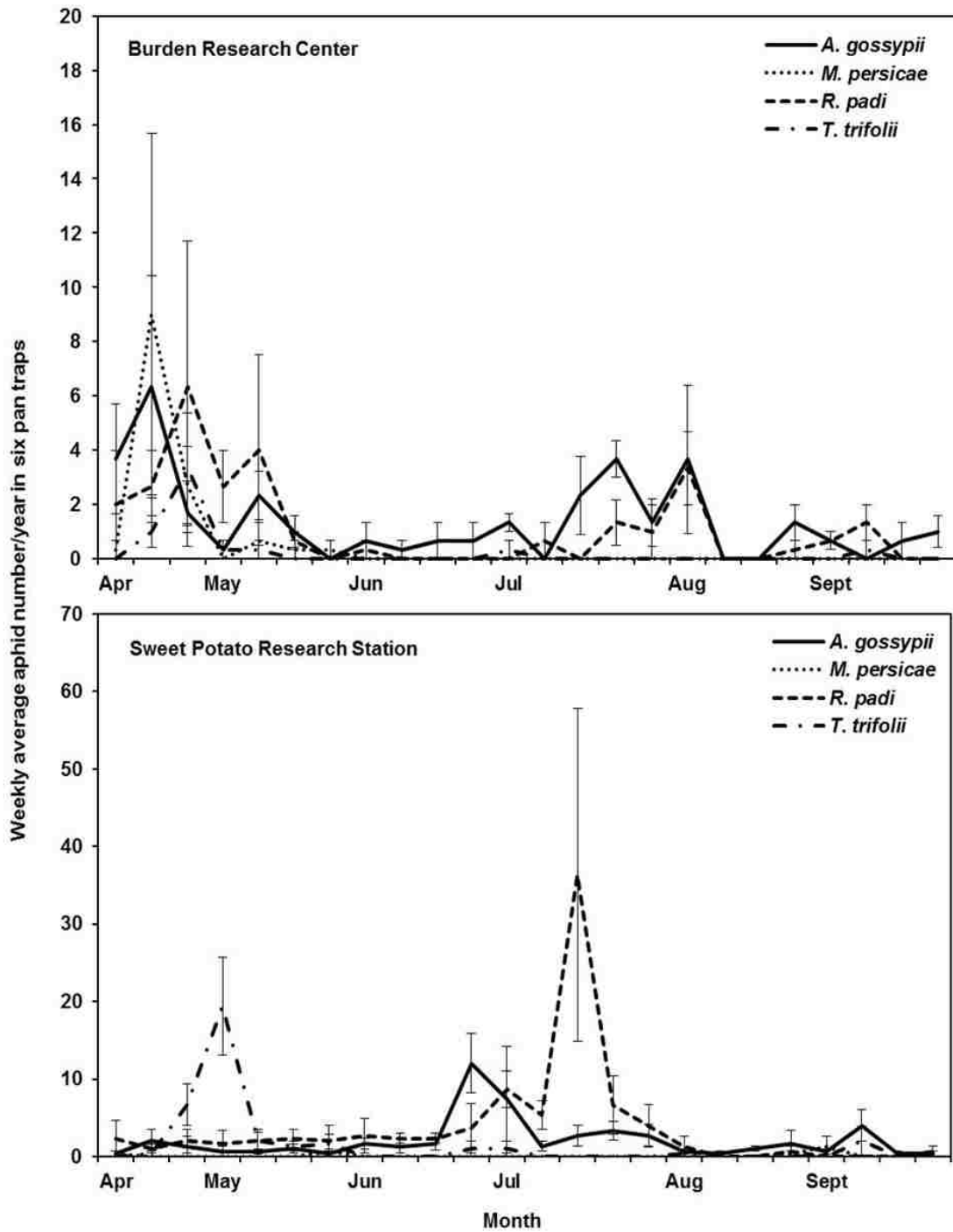


Fig. 4.4. Weekly average number of the four most dominant aphid species, *Aphis gossypii*, *Myzus persicae*, *Rhopalosiphum padi*, and *Therioaphis trifolii*, captured in pan traps (total number for six pan traps) at Burden Research Center and Sweet Potato Research Station(2009 to 2011) (Means \pm SE).

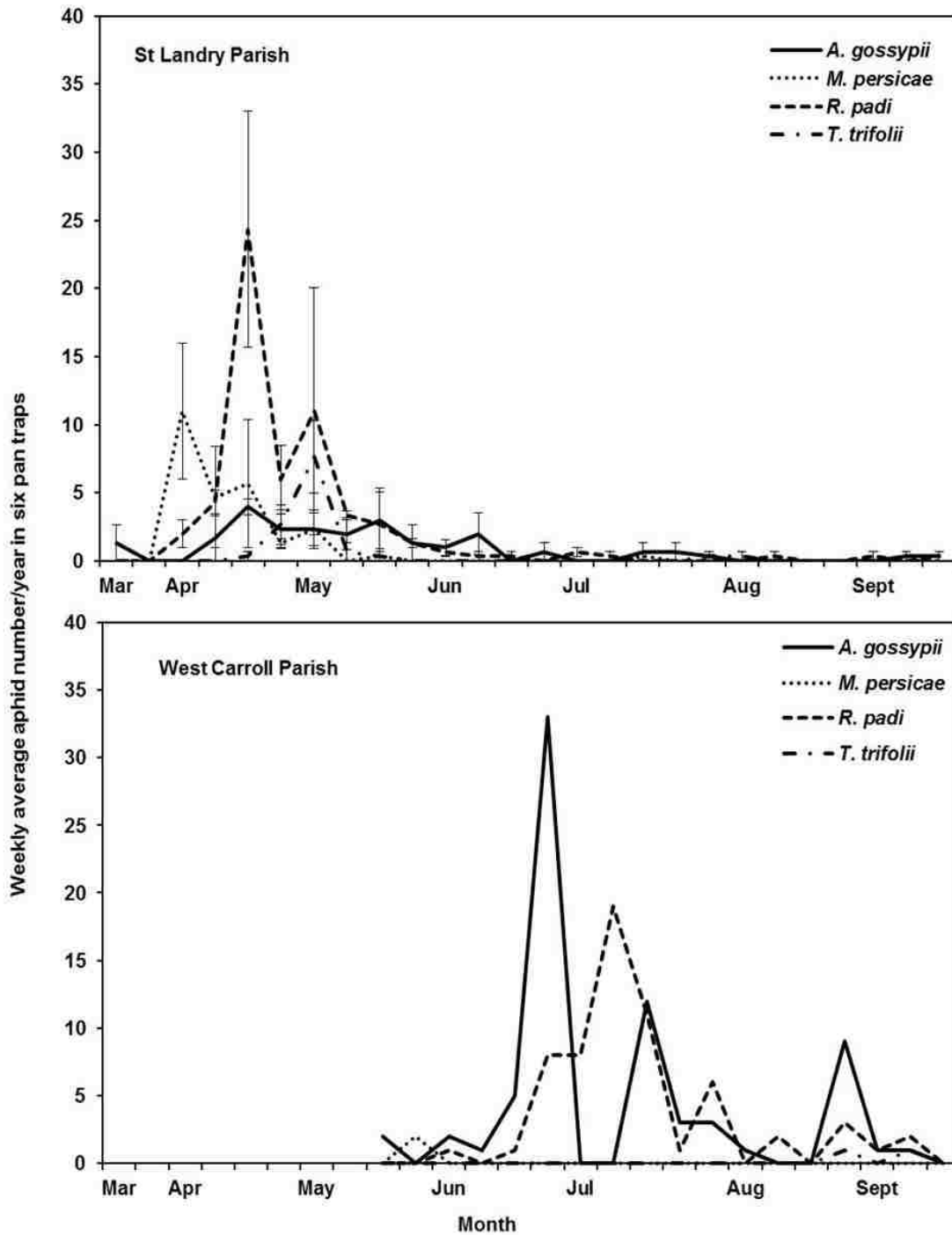


Fig. 4.5. Weekly average number of four most dominant aphid species, *Aphis gossypii*, *Myzus persicae*, *Rhopalosiphum padi* and *Therioaphis trifolii*, captured in pan traps (total number for six pan traps) at St Landry in 2009 to 2011 (Means \pm SE) and West Carroll (2009) parishes.

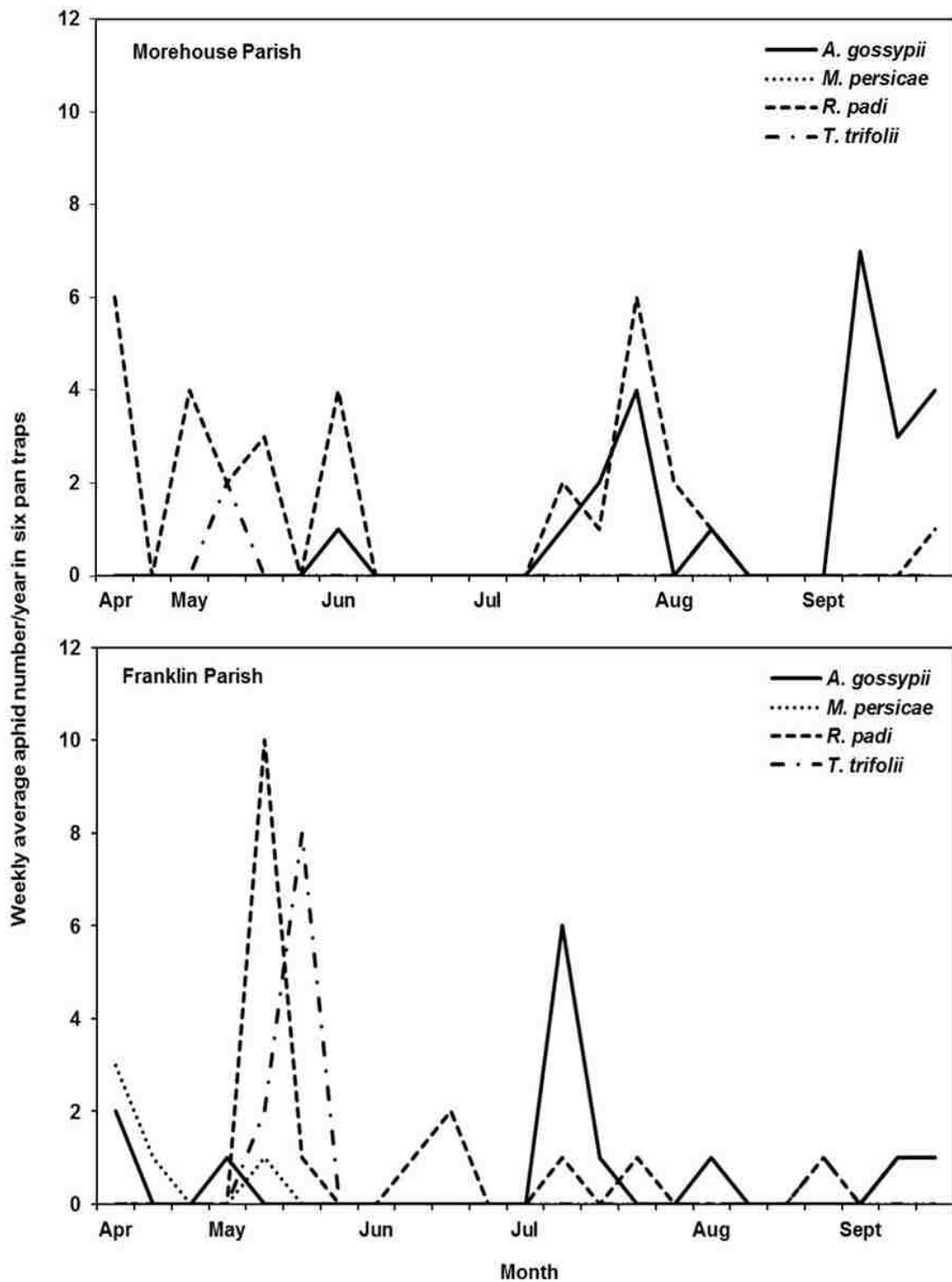


Fig. 4.6. Weekly average number of the four most dominant aphid species, *Aphis gossypii*, *Myzus persicae*, *Rhopalosiphum padi*, and *Therioaphis trifolii*, captured in pan traps (total number for six pan traps) at Morehouse (2010) and Franklin (2011) parishes.

4.3.3 Virus titer quantification

Titers of SPFMV in sweetpotato were generally higher during the period of late June to September when plants were undergoing rapid vine growth in the field compared to May to mid-June when plants were in plant beds or just newly transplanted to the field (Fig. 4.9). *Sweet potato virus G* titers varied greatly within and between seasons. They were greatest during late May and again in late August during 2010 and in June during 2011 (Fig. 4.10). *Sweet potato virus 2* titers were greater during the period of early June and August during 2010, and during July in 2011 (Fig. 4.11). Correlation analysis between SPFMV titers and infection of sentinel plants at sites in Louisiana revealed a significant positive relationship ($r = 0.45$; $P = 0.0337$). Correlation analysis with SPV2 showed a non-significant positive relationship ($r = 0.27$; $P = 0.2863$), while SPVG had a significant negative relationship ($r = -0.52$; $P = 0.0286$).

4.3.4 Morning glory weeds

A majority of the morning glory plants observed in sweetpotato plant beds and fields were annuals that germinated from seed mainly between May to July. The most common species at all study fields were *I. hederacea* and *I. cordatotriloba* that occurred in the range of about 1 to 5 seedlings/20 m². *Ipomoea lacunosa* was observed only at the Morehouse parish. The St Landry parish site had a high population of *J. tamnifolia* (up to about 10 seedlings per 1 m² in non-cultivated plowed areas around the sweetpotato fields) throughout the three year period, while the other sites had very few plants of this species. Each year, although morning glories were present both in beds and fields, virus symptoms were not observed on those growing in or near beds at all study sites.

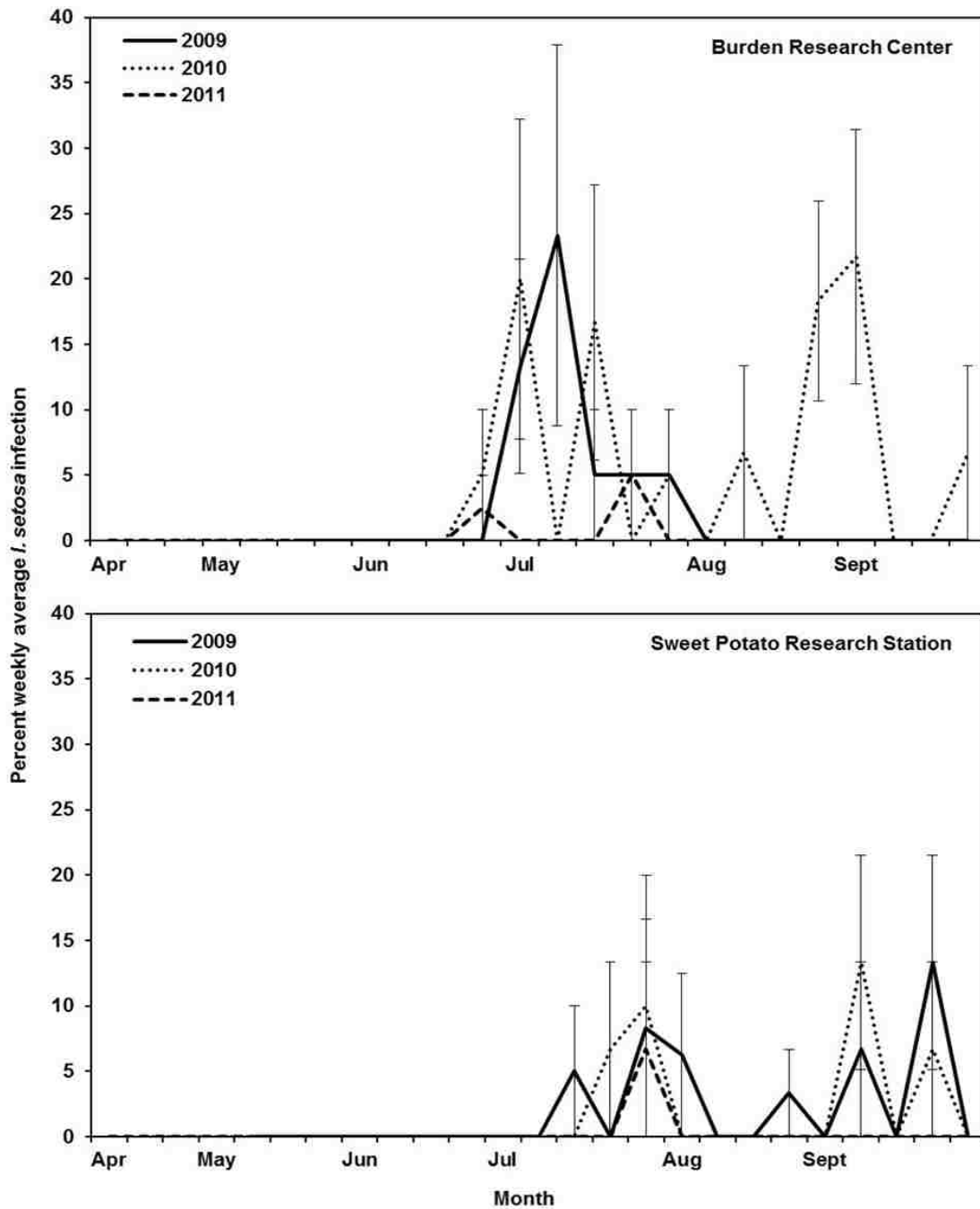


Fig. 4.7. Weekly average percent infection (means/field) of *Ipomoea setosa* sentinel plants by sweetpotato potyviruses in sweetpotato fields at Burden Research Center and Sweet Potato Research Station (Means \pm SE).

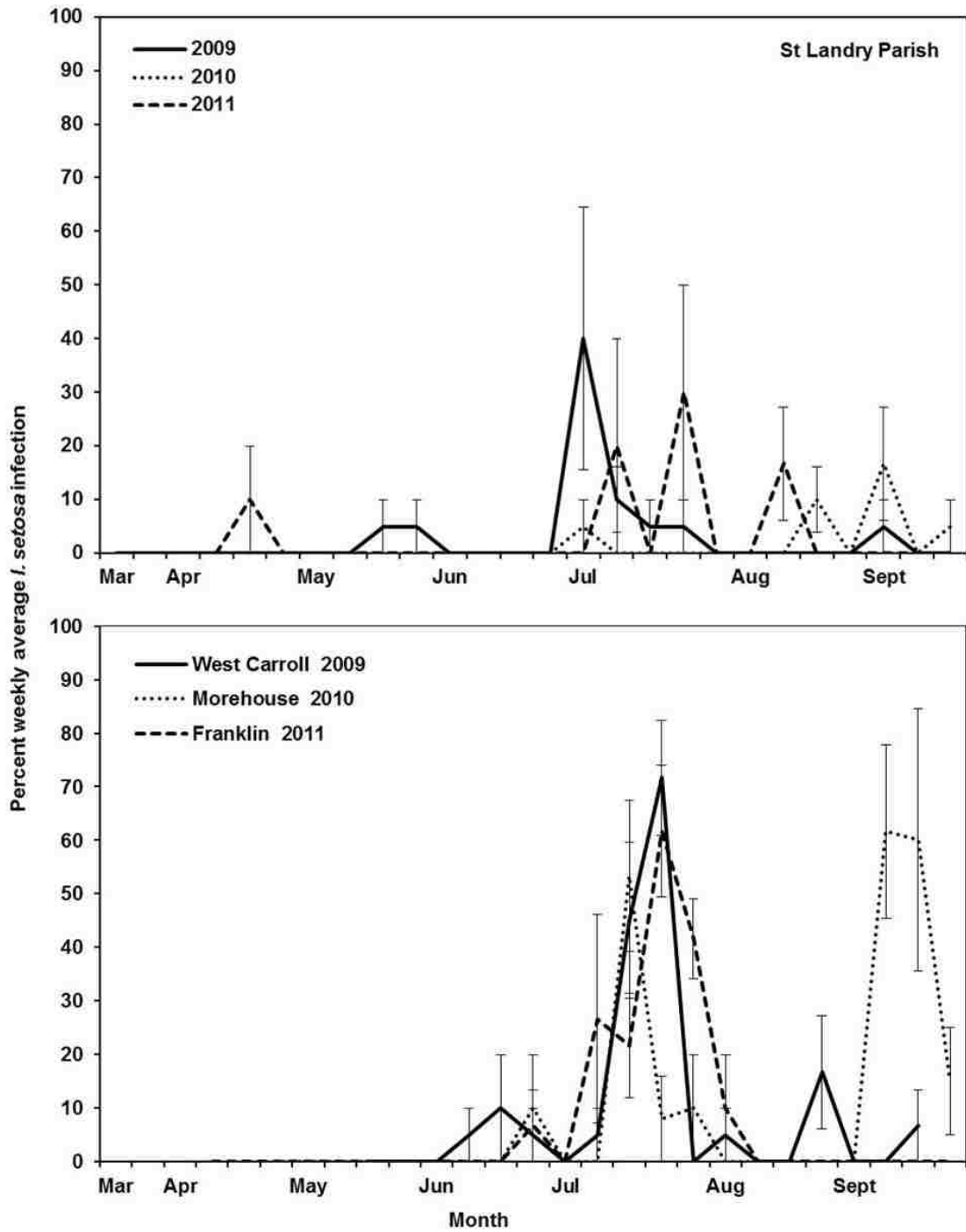


Fig. 4.8. Weekly average percent infection (means/field) of *Ipomoea setosa* sentinel plants by sweetpotato potyvirus in sweetpotato fields at St Landry (2009 to 2011), West Carroll (2009), Morehouse (2010) and Franklin (2011) parishes (Means \pm SE).

Table 4.2. Number of *Ipomoea setosa* plants exposed in sweetpotato fields, incidence of potyvirus symptoms and incidence of specific potyviruses as determined by NCM-ELISA in Louisiana 2009 to 2011.

Year	Plants exposed (No)	Plants with symptoms (No)	% total infected ^z	% SPFMV ^y	% SPVG ^y	% SPV2 ^y
2009	1394	66	5	97	9	6
2010	1166	55	5	100	10	1
2011	1129	39	4	100	15	3

^z The percentage of all sentinel plants exposed that developed potyvirus symptoms.

^y The percentage represents those plants that were symptomatic and tested positive for the respective virus species either in single or mixed infections. SPFMV = *Sweet potato feathery mottle virus*, SPVG = *Sweet potato virus G*, and SPV2 = *Sweet potato virus 2*.

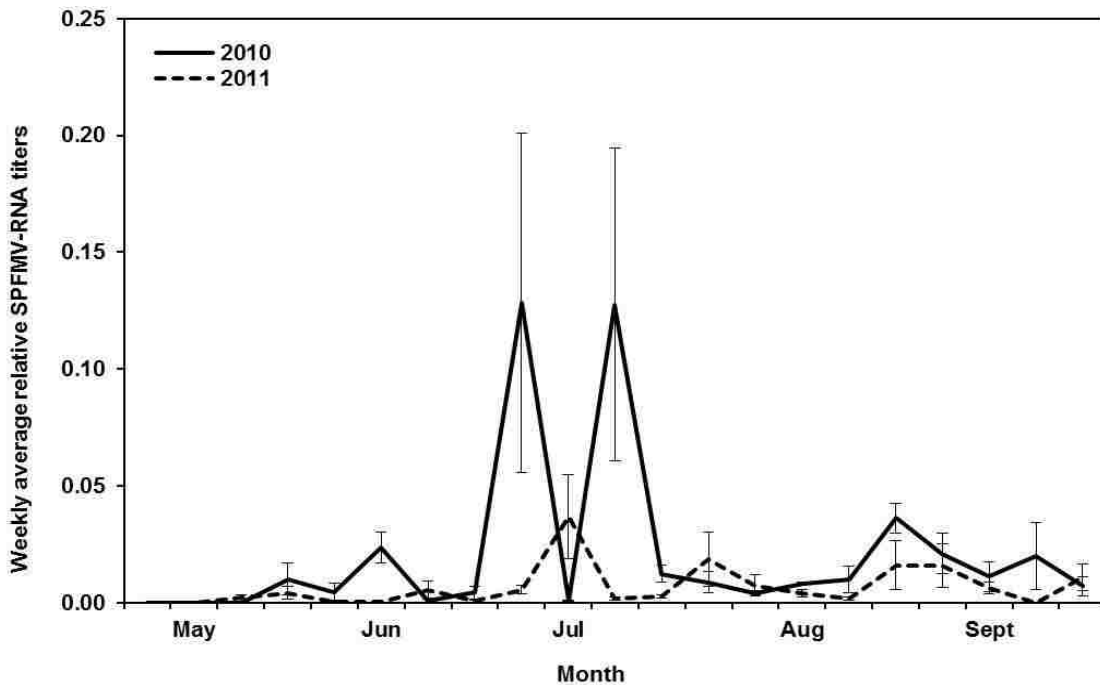


Fig. 4.9. Average relative *Sweet potato feathery mottle virus* RNA titers in Beauregard sweetpotato leaves sampled weekly at Burden Research Center in 2010 and 2011 (Means ± SE).

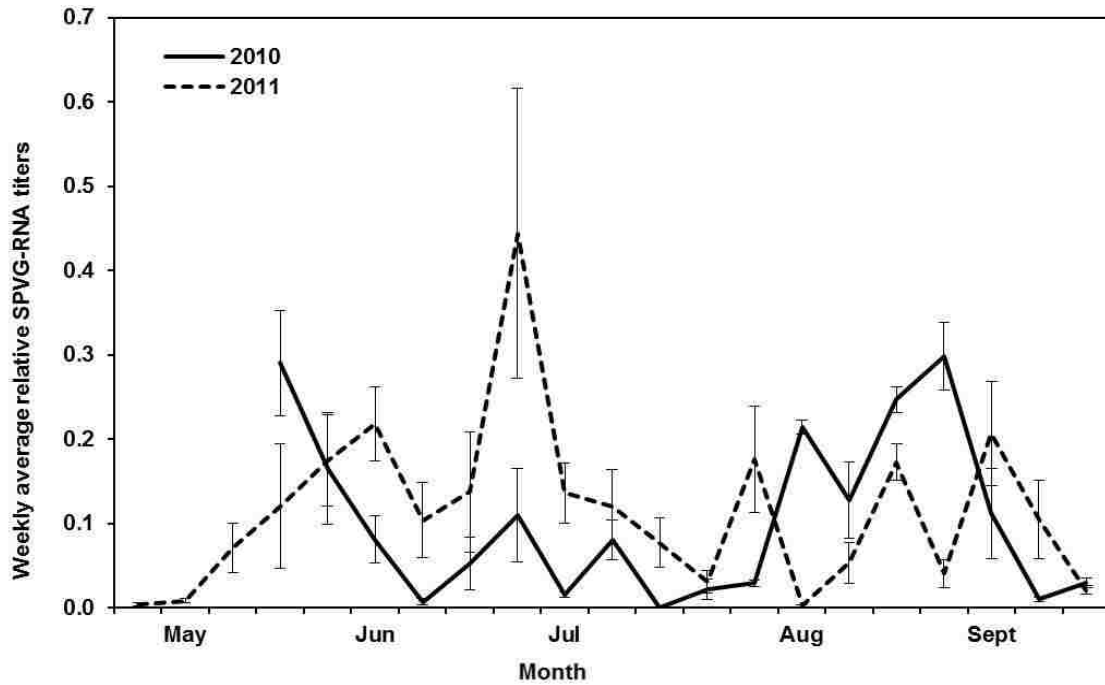


Fig. 4.10. Average relative *Sweet potato virus G* RNA titers in Beauregard sweetpotato leaves sampled weekly at Burden Research Center in 2010 and 2011 (Means \pm SE).

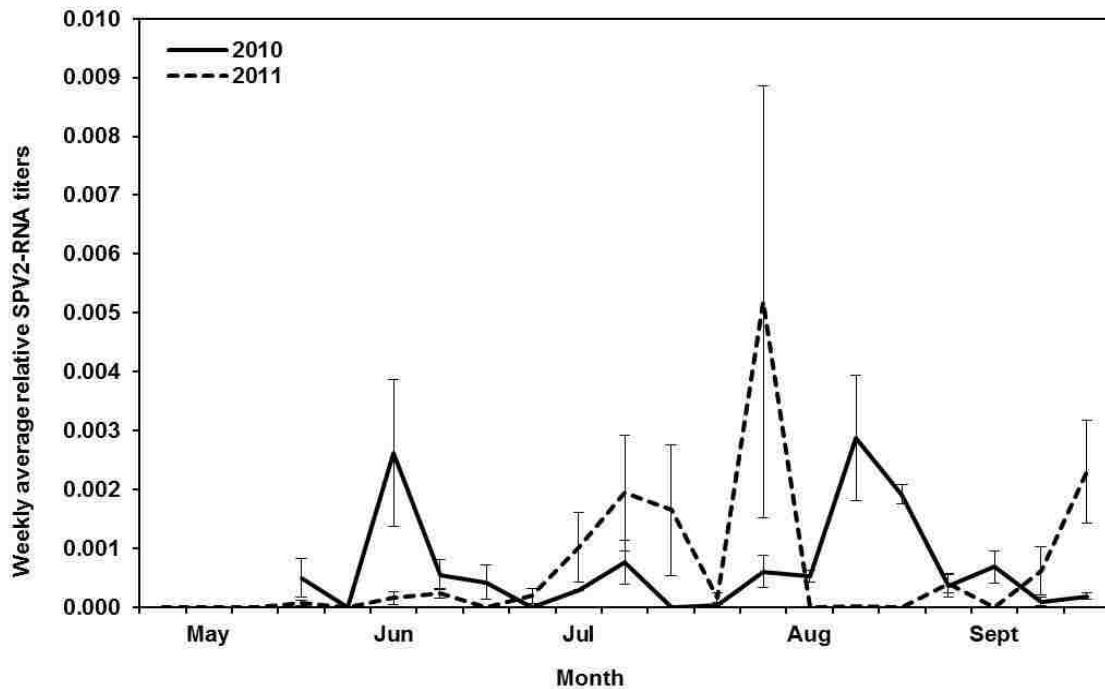


Fig. 4.11. Average relative *Sweet potato virus 2* RNA titers in Beauregard sweetpotato leaves sampled weekly at Burden Research Center in 2010 and 2011 (Means \pm SE).

Virus symptoms on morning glories were first observed on *I. hederacea* and *I. cordatotriloba* plants during the period of July at most sites while *J. tamnifolia* remained asymptomatic at these sites throughout the season. At St Landry in 2009, 2010 and 2011 the first virus symptoms on morning glories were observed on 16th of July (*I. cordatotriloba*), 15th of July (*I. hederacea*) and 22nd of July (*I. cordatotriloba*) respectively. At Morehouse parish in 2010 the first virus symptoms on morning glories (*I. hederacea*) were observed on 28th of July. At Franklin parish in 2011, morning glories were not observed within sweetpotato fields. They were mostly along the hedges and drainage channels which were more than 10 meters from sweetpotato fields, and no symptoms virus symptoms were observed on them. Virus symptoms were not observed on morning glories that were found growing in sweetpotato fields at the Sweet Potato Research Station during the three year period except in 2009 when only one plant growing along the fence showed virus symptoms during the month of August. Virus symptoms were not observed on morning glories growing both in beds and in the field at Burden Research Center throughout the study period.

4.3.5 Virus vector testing

Rhopalosiphum maidis and *R. padi* successfully transmitted SPFMV from a singly infected *I. cordatotriloba* (Table 4.3).

Table 4.3. Sweet potato feathery mottle virus transmission from *Ipomoea cordatotriloba* to *I. nil* cv. Scarlet O'Hara by *Rhopalosiphum maidis*, *Rhopalosiphum padi* and *Myzus persicae* following 1 minute acquisition probes.

Aphid species	% transmission	
	Mean	95% Confidence Interval
<i>M. persicae</i>	22	14.7 – 30.9
<i>R. maidis</i>	1	0.0 – 4.7
<i>R. padi</i>	2	0.4 – 6.4
Control	0	NT

n = 100 for each acquisition source

Means within a column (Aphid species) with an overlapping CI range are not significantly different (P>0.05).

(NT) No transmission occurred therefore no confidence intervals can be calculated.

4.4 Discussion

Despite the fact that aphids were present throughout the entire growing season, sweetpotato potyviruses were not transmitted to *I. setosa* sentinel plants to any significant extent in plant beds, where density and proximity of susceptible plants is greatest.

Instead, transmission occurred primarily during a period after transplanting to production fields when the sweetpotato vines were growing rapidly. Aphid numbers and species composition varied during the season and were not correlated with virus transmission to sentinel plants. In fact, the period of greatest virus spread began in late June to early July, between the first and second peaks of aphid flight which occurred in mid-April to early June, and mid-August to early September, respectively.

Lack of significant differences in aphid captures based on trap location within each field suggests that their flight over sweetpotato fields is relatively uniform, indicating they are migratory transients that originate from other crops or plants and probably spread viruses uniformly within the field. The first peaks of aphid flight could be attributed to migration of alates from their overwintering hosts, while the second could be related to redistribution of aphids due to deteriorating host quality in the

landscape from crops such as wheat, corn, soybean and cotton, and weedy species such as smell melon, nightshade and Johnson-grass.

Aphid species were diverse and varied in percent composition among years and within each season. Despite a large number of species captured, a few species (3 to 7) accounted for over 83% of the population in any given year at all sites. The most common species were *A. gossypii*, *M. persicae*, *R. padi* and *T. trifolii*. Earlier studies by Kantack et al. (1960) in south Louisiana sweetpotato fields where aphids were trapped using tangle foot traps, found mainly *A. gossypii*, *M. persicae* and *M. euphorbiae*. *Aphis gossypii* and *M. persicae* are the most efficient vectors of sweetpotato potyviruses of those species evaluated so far (Wosula et al., 2012). *Aphis gossypii* was captured at all locations and was also present in the fields during most of the growing season. *Myzus persicae* was captured only during the months of April to May when little virus transmission was recorded. *Rhopalosiphum padi* was present at all locations and occurred throughout the growing season with peaks in April to May, and in July. Whether *T. trifolii* can transmit sweetpotato viruses is unknown, but it was present at most locations, mainly during the months of April to May. Although the number of aphids in yellow pan traps was higher compared to green pan traps, most of the species were captured in both traps. This indicates that in our case, using yellow traps provided a better indication of aphid numbers but not necessarily species diversity.

Several authors have reported similar fluctuations of aphid population abundance and changes in species composition depending on season, year and location (DiFonzo et al., 1997; Thomas et al., 1997). These fluctuations could be attributed to biotic factors such as aphid reproduction cycle, host plant quality and availability, predators,

parasitoids and entomophagous fungi or abiotic factors such as rainfall, temperature, wind and light intensity (Dixon, 1998; Michaud, 2010). Pest management practices such as the use of insecticides also affect development and survival of insects (Kennedy and Storer, 2000; Kerns and Gaylor, 1993). The pattern of aphid flights in our study could have been affected by these biotic and abiotic factors because populations fluctuated with season. Aphids in rare cases do colonize sweetpotato, *M. persicae* and *M. euphorbiae* have been observed colonizing sweetpotato in Louisiana (Davis, J. A. personal communication). In 2009, *M. euphorbiae* heavily colonized sweetpotato plants in beds at the St Landry site (up to 30 aphids/plant on Beauregard, and 19 aphids/plant on Evangeline) prior to application of an aphicide, but no aphids were observed colonizing at other sites. This could be the reason why this species was among the dominant species in 2009 but was very low or absent in other years. The St Landry site in south Louisiana is under a mandatory spray program for controlling sweetpotato weevils. Certain insecticides are known to induce aphid outbreaks, and this may have contributed to colonization and rapid population increase by *M. euphorbiae* in these beds (Kerns and Gaylor, 1993). Sweetpotato plant beds and production fields were scouted regularly for colonizing aphids, and none were observed. Therefore, most of the aphids were transient non-colonizing migrants that originated from crops or vegetation surrounding sweetpotato fields, since only alates were captured on sticky traps or in pan traps. Non-colonizing species are typically more important in spreading non-persistent viruses in a crop (Racah et al., 1985). This is because non-colonizing aphids are more likely to probe epidermal leaf cells and disperse rather than settle, hence increasing the likelihood of virus acquisition and transmission (Nault and Bradley, 1969). Although both A.

gossypii and *M. persicae* are known efficient vectors of sweetpotato potyviruses, the latter seems to play a lesser role in spread of these viruses in the field. *Myzus persicae* was found only during a short period when plants were still in plant beds, a trend also observed in a previous study (Kantack et al., 1960), and a period when sentinel plants were not infected. Basky et al. (2001) observed *M. persicae* early in the season and suggested it could play a role in early transmission of *Zucchini yellow mosaic virus* (ZYMV) in squash, and we cannot rule out the possibility that low levels of virus spread may occur in beds during this period. *Aphis gossypii* was consistently present during periods when potyviruses spread in the sweetpotato fields, a phenomenon that was also observed by Kantack et al. (1960). Furthermore, laboratory studies of SPFMV transmission from sweetpotato and morning glory plants indicate that *A. gossypii* is a more efficient vector compared to *M. persicae* (Wosula et al., 2012). Abundance and transmission efficiency are two major factors that determine vector propensity (Davis et al., 2008). Although *R. padi* was a less efficient vector of SPFMV under laboratory conditions in this study, it was present in large numbers and may also have contributed to the spread of SPFMV as has been observed with *R. padi* and other potyviruses on other hosts (Banik and Zitter, 1990; Basky et al., 2001; Davis et al., 2008). This is believed to be the first report of transmission of SPFMV by *R. maidis* and *R. padi* although they are less efficient vectors. The other common species, *T. trifolii*, whose status as a vector of sweetpotato potyviruses is not known, is a vector of *Potato virus Y* (PVY), *Cucumber mosaic virus* (CMV) and *Bean yellow mosaic virus* (BYMV) (Berlandier et al., 1997; DiFonzo et al., 1997). Most of the other aphid species captured in this study have not been assessed for their ability to transmit potyviruses and should be evaluated.

Transmission by *R. maidis* and *R. padi* should also be evaluated under field conditions. These aphids were very restless during handling, a behavior that was not observed in *M. persicae* under laboratory transmission experiments, which may have contributed to the less efficient transmission of SPFMV.

Although sentinel plants were placed at sites throughout the study period, major infections occurred beginning late June to August at all locations. Most infections occurred during a period of relatively low total aphid populations except at Morehouse where high aphid populations coincided with infections in sentinel plants. Contrary to the other studies which correlate aphid abundance and spread of non-persistent viruses in crops (Basky et al., 2001; DiFonzo et al., 1997), we found that virus spread in Louisiana was not correlated with aphid abundance except at Morehouse parish. Other factors, such as source and concentration of inoculum may also be involved in non-persistent virus epidemics (Raccah et al., 1985). In this study, as in a previous study (Kantack et al., 1960), SPFMV transmission was greatest in the field a few weeks after the crop was transplanted when vines were growing most vigorously. SPFMV titers were low during early season when plants were still in plant beds or had just been transplanted to the field. However, the titers increased in plants 3 to 4 weeks after transplanting to the field when vines had begun to grow rapidly and maximum titers occurred during the months of June to August. Symptoms followed a similar trend to virus titers: plants in the beds were asymptomatic most of the period and only developed mild symptoms of vein chlorosis and yellow chlorotic spots from early June to the first week of July, when transplanting the crop was ending. During the second week of July to mid-August, plants displayed typical symptoms of purple and yellow chlorotic spots, and vein chlorosis. During mid-

August to mid-September only lower and middle leaves had diffuse purple discolorations, while the upper leaves were asymptomatic. The above described symptoms are typical of naturally infected sweetpotato plants (i.e. grown in the field for several years) which test positive for these three potyviruses and negative for other known sweetpotato viruses (Clark and Hoy, 2006; Clark et al., 2010), possibly indicating the presence of other unknown viruses. Several authors have reported fluctuation in virus titers with cropping season. These fluctuations are attributed to climatic changes, especially temperature and the physiological status of host plants (Dovas et al., 2002; Torrance and Dolby, 1984).

The apparent association of higher SPFMV virus titers in late June to August with the period of peak transmission in the field relates well to laboratory transmission studies of SPFMV in which higher transmission rates were obtained from sources with higher SPFMV (Wosula et al., 2012). This suggests that virus titer levels can be a limiting factor to virus spread. Other authors have also observed increased transmission of virus from sources with higher titers compared to those with low titers; for example *Watermelon mosaic virus* (WMV) and CMV in muskmelon (Banik and Zitter, 1990), and PVY in potato (De Bokx et al., 1990). Although morning glory seedlings were present in plant beds, none were observed to develop symptoms during the time transplants were being cut from the beds. Virus symptoms appeared on *I. hederacea* and *I. cordatotriloba* plants in fields during the month of July, about 6 to 8 weeks after sweetpotato plants were transplanted to the field or about 2 to 3 weeks after initial sentinel plant infections were observed. Since 1 to 2 weeks is required for these viruses to induce symptoms in these hosts, it suggests that morning glory plants were not the primary sources of inoculum but probably were also infected by the inoculum that infected the sweetpotatoes. Based on

our study, the results suggest previously infected sweetpotato among field plants is the source of primary inoculum. However, the morning glories may serve as better sources of secondary inoculum that might extend the period of virus spread into later field stages since they develop higher titers of the virus and support greater levels of SPFMV transmission than sweetpotatoes in greenhouse conditions (Wosula et al., 2012). This can be assessed further by quantifying virus titer in morning glory plants compared to sweetpotato under field conditions. The role of *J. tamnifolia* is not clear as symptoms were not observed on seedlings in this study although infected plants have been found previously in sweetpotato fields (Clark, C. A. personal communication). Other weeds have also been reported to be better virus sources than the crops with which they co-exist. For example hairy nightshade is a better source of *Potato leafroll virus* (PLRV) compared to potato (Alvarez and Srinivasan, 2005).

Previous studies indicated that after one generation in the field, sweetpotato plants are commonly infected with SPFMV but by the third to fourth generation, incidence of SPFMV was 100%, SPVG was 50 to 70% and SPV2 was 25 to 30% (Clark et al., 2010). In this study, SPFMV was present in 90 to 100% of all symptomatic sentinel plants, followed by SPVG (9 to 25%) and SPV2 (1 to 28%). These results indicate SPFMV is transmitted far more efficiently and is the dominant potyvirus spreading in Louisiana sweetpotatoes.

The preferential transmission of SPFMV despite its occurrence in mixed infections with SPVG and SPV2 could in part be due to its increased frequency in sweetpotato plants. *Aphis gossypii* transmits SPFMV preferentially than SPVG from mixed infected sources under laboratory conditions (Wosula et al., 2012), but both were

transmitted efficiently from singly infected source plants (Souto et al., 2003).

Preferential transmission of SPFMV could also be due to competition for receptor sites in the aphid vectors, which may become saturated with SPFMV giving higher transmission rates (Power, 1996). SPFMV was originally differentiated into four strains: russet crack (RC), common (C), ordinary (O) and East African (EA) (Kreuze et al., 2000). However, the C strain was separated in 2010 into a distinct species, *Sweet potato virus C* (SPVC), due to its nucleotide sequence divergence from the other three strains (Untiveros et al., 2010). Since the antiserum used for SPFMV detection in NCM-ELISA and the primer/probe set used in qRT-PCR for virus quantification do not differentiate SPFMV and SPVC, our use of SPFMV in this study reflects the original definition of this virus species (Moyer and Salazar, 1989) and includes both the SPFMV and SPVC *sensu* Untiveros et al. (2010). When methods are available to differentially quantify SPFMV and SPVC, their roles in the sweetpotato potyvirus complex will need to be re-evaluated.

In the USA, potyviruses have been managed through reducing virus inoculum by using limited generation seed that is initially virus free, and continually flushing out the diseased material (Clark et al., 2012). Despite this effort, sweetpotato plants in Louisiana fields are frequently re-infected at high rates with the predominant potyviruses. Results from this study can be used to help design additional management strategies to reduce re-infection of virus tested material. These strategies may include limiting availability of primary and secondary inoculum by separating beds and fields, separating seed plots from commercial crop and control of weedy morning glories. Others include study on use of compounds such as mineral oils that may reduce spread of viruses during peak periods and also use of barrier crops. Little can be done to control the aphid vectors since

most of them are transient migrants that originate from other crops and plants in the landscape and not sweetpotato. Furthermore, use of insecticides to control aphids has not reduced spread of non-persistent viruses because of the very short period required for their acquisition and inoculation (Davis et al., 2008). Morning glory plants in sweetpotato fields were mostly annuals that showed virus symptoms concurrently with the sentinel plants, suggesting that sweetpotato itself is the major source of primary inoculum in commercial production fields. Earlier studies by Clark et al. (2010) reveal that most of the sweetpotato plants grown out in growers' fields in Louisiana are already infected with potyviruses. Although morning glory plants may not be sources of primary inoculum, they could act as sources of secondary inoculum once infected, potentially providing a high-titer source of virus after titers have declined in the sweetpotatoes. Future research should evaluate the potential role of such morning glory populations in potyvirus epidemiology to determine if using fields with minimal morning glory populations can help reduce re-infection of sweetpotato 'seed' root crops.

Based on these results, growers could establish their field plantings beginning with the furthest fields from plant beds especially if they intend to have multiple cuttings taken from the beds. This will minimize rapid infection of plants still in beds with abundant inoculum in field plants whose virus titer levels increase rapidly 3 to 4 weeks after transplanting. Seed production fields should also be located away from any other sweetpotato plants especially of previous generations that could act as sources of inoculum. The plots for foundation seed should be located away from any possible source of virus inoculum. Davis et al. (2008) suggests isolation from sources of inoculum as one of the cultural practices to minimize spread and infection of viruses.

Several strategies have been used in other crop systems to minimize spread of non-persistent viruses. Although resistance has been used to manage several sweetpotato diseases, resistance to potyviruses and/or their vectors has not been evaluated and could present a long term strategy to minimizing losses (Davis et al., 2008). Other strategies include use of mineral oil sprays, use of reflective mulches and use of barrier or protector plants (Davis et al., 2008). Further studies should be carried out to determine whether these and other strategies can be used to minimize virus spread in the sweetpotato system. This study has identified the critical period of the sweetpotato crop when such management strategies could best be deployed to reduce infection with SPFMV. This study revealed that inoculum availability and concentration plays an important role in virus spread, any new strategies established to minimize virus spread could therefore only work well when accompanied with controlled inoculum concentrations through use of virus tested/clean propagation material.

CHAPTER 5: POPULATION DYNAMICS OF THREE APHID SPECIES ON FOUR *IPOMOEA* SPP. INFECTED OR NON-INFECTED WITH SWEETPOTATO POTYVIRUSES

5.1 Introduction

Sweetpotato [*Ipomoea batatas* (L.) Lam.], a member of the family *Convolvulaceae*, is widely cultivated in tropical and sub-tropical areas and ranks among the top 10 most important food crops worldwide (Loebenstein and Thottappilly, 2009). Sweetpotato is vegetatively propagated and is prone to accumulate viruses which cause cultivar yield decline and reduce storage root quality (Clark and Hoy, 2006; Clark et al., 2010). Of the sweetpotato viruses so far described, the most common worldwide is *Sweet potato feathery mottle virus* (SPFMV) (Clark et al., 2012). In the USA, the most commonly recognized viruses are the potyviruses: *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato virus G* (SPVG) and *Sweet potato virus 2* (SPV2, synonym = *Ipomoea vein mosaic virus*) (Clark et al., 2012). *Sweet potato virus C* (SPVC) a distinct species which was formerly the common strain of SPFMV (Untiveros et al., 2010) may also be prevalent since it has been detected among field isolates that were previously obtained through natural aphid inoculation of sentinel plants (*Ipomoea setosa*), and maintained in sweetpotato cv. Beauregard in the greenhouse (Clark, C. A. unpublished) using the newly developed multiplex RT-PCR technique (Li et al., 2012)

Sweetpotato potyviruses commonly occur as mixed infections in the field (Clark et al., 2010; Souto et al., 2003). Mixed infections of SPFMV, SPVG and SPV2 in sweetpotato cv. Beauregard usually show mild chlorotic spotting and veinal chlorosis or no symptoms, and cause yield losses of less than 15% (Clark et al., 2010). Sweetpotato potyviruses also infect other members of the morning glory family, many of which occur

as wild plants or weeds in cultivated fields. In Louisiana the most common morning glory species within or around sweetpotato fields are *Ipomoea cordatotriloba* Dennst. (synonym *I. trichocarpa* Ell., cotton morning glory) and *I. hederacea* Jacq. (ivy-leaf morning glory) (Clark et al., 1986).

Efficient field transmission of these viruses is mainly by several aphid species in a non-persistent manner (Kennedy et al., 1962). *Myzus persicae* is one of the known efficient vectors of these viruses (Wosula et al., 2012) and is among the dominant aphids captured in Louisiana and Mississippi sweetpotato fields (Wosula et al., 2012). Other commonly caught aphid species include *Aphis gossypii* (an efficient vector) and *Rhopalosiphum padi* (a less efficient vector) (Wosula et al., 2012). These three aphid species comprise approximately 70% of the aphids landing in sweetpotato fields in the Mid-South.

Plant-mediated interactions between pathogens and arthropods are determinants of population dynamics in managed and natural ecosystems (Stout et al., 2006). Plant pathogen-vector system interactions can have both direct and indirect effects. The possible components through which the systems could interact include: dependence of the virus on the arthropod vector for transmission, pathogen effect due to its presence and replication in the vector, pathogen and vector competition for limiting resources, and pathogen and vector potential to induce host defense mechanisms hence affecting each other indirectly through the response of the plant (Belliere et al., 2005). Plant viruses can alter plant quality and physiology in ways that can either be beneficial (Belliere et al., 2005; Blua and Perring, 1992; Maris et al., 2004; Srinivasan et al., 2008), neutral (Hodge and Powell, 2008; Roca et al., 1997; Wijkamp et al., 1996) or detrimental (Donaldson

and Gratton, 2007; Hodge and Powell, 2008; Jiménez-Martínez and Bosque-Pérez, 2009; Mauck et al., 2010; Michels et al., 1994) to vector growth rates, reproduction, longevity and preference. Increased performance of sucking insects on virus-infected plants, for example, is often correlated with increases in free amino acids and soluble sugars in the phloem sap (Ajayi, 1986; Blua et al., 1994; Fereres et al., 1990). Reduced performance could be attributed to poor host quality due to severe virus infection, causing decline in availability of resources due to reduced plant photosynthesis and growth (Gao and Nassuth, 1993; Hodge and Powell, 2008).

Vector biology and ecology is, in most cases, neglected when dealing with virus epidemiology (Radcliffe and Ragsdale, 2002). Aphids reported to colonize sweetpotato include *Aulacorthum solani* (Kalt.), *A. gossypii*, *Aphis nasturtii* (Kalt.), *Aphis spiraecola* (Patch), *Macrosiphum euphorbiae* (Thomas) and *M. persicae* (Blackman and Eastop, 2006), but their reproductive behavior on this crop is unknown. Since most sweetpotato plants in Louisiana growers' fields are infected with potyviruses (SPFMV= 100%, SPVG = 50 to 70%, SPV2 = 25 to 30%) (Clark et al., 2010), there is a high probability that colonizing aphids will encounter virus-infected plants. In addition, SPFMV infects weedy morning glory plants in Louisiana (Clark et al., 1986) but its percentage incidence in these plants is unknown. Tugume et al. (2008) reported that in Uganda 58 to 62% of weeds belonging to the family *Convolvulaceae* expressing virus-like symptoms and in close proximity with sweetpotato tested positive for SPFMV. Knowledge on how these viruses affect aphid performance is essential in order to predict aphid population dynamics and progression of virus epidemics within sweetpotato fields. The objective of this study was to (i) determine if *A. gossypii*, *M. persicae*, and *R. padi* can utilize

sweetpotato cv. Beauregard with mixed virus infection as a host plant, and if so, (ii) determine reproductive behavior of each aphid on virus-infected and non-infected sweetpotato cvs. Beauregard and Evangeline, and morning glory species *I. cordatotriloba* and *I. hederacea* by conducting life table analyses.

5.2 Materials and methods

5.2.1 Host plants

To ensure that plants were initially free of viruses, sweetpotato plants were derived from virus-tested mericlones maintained by nodal propagation in tissue culture at the LSU AgCenter Department of Plant Pathology and Crop Physiology and *Ipomoea cordatotriloba* and *I. hederacea*, were established from seeds harvested from single plants that were grown in the greenhouse. All plants were grown in the greenhouse under wide temperature (20-32°C) and humidity (21-98%) ranges, in 10 cm diameter clay pots containing autoclaved soil mix consisting of 1 part river silt, 1 part sand, 1 part Jiffy-Mix Plus (Jiffy Products of America Inc.) and 3.5 g/pot Osmocote 14-14-14 (Scotts-Sierra Horticultural Products Company). Plants were not sprayed with insecticide.

5.2.2 Virus inoculum

The russet crack strain of SPFMV (SPFMV-RC, isolate 95-2) was maintained in *I. nil* cv. Scarlet O'Hara (SOH) in the greenhouse by repeated mechanical inoculation, and a naturally mixed infected sweetpotato cv. Beauregard (B 14, G-7) that was grown in fields in North Carolina for seven years and provided by G. C. Yencho (Dept. Horticultural Sciences, North Carolina State University, Raleigh) was maintained by vegetative propagation. B 14, G-7 was tested using RT-PCR and qRT-PCR and after grafting on *I. setosa* using NCM-ELISA, and found to be infected with SPFMV, SPVG

and SPV2, but tested negative for *Sweet potato mild mottle virus*, *Sweet potato latent virus*, *Sweet potato chlorotic fleck virus*, *Sweet potato mild speckling virus*, *Sweet potato leaf curl virus*, *Sweet potato chlorotic stunt virus*, *Sweet potato collusive virus*, and *Cucumber mosaic virus*. However, the possibility that it was infected by viruses not yet recognized in sweetpotato cannot be eliminated. Recently, with the separation of the common strain of SPFMV into a distinct virus species *Sweet potato virus C* (SPVC) (Untiveros et al., 2010), B14, G-7 was tested using the newly developed multiplex RT-PCR technique (Li et al., 2012) and was found to be also infected with SPVC.

5.2.3 Aphid colony

Myzus persicae was collected from an unknown host in 2004. *Aphis gossypii* was collected from cotton at the LSU AgCenter Macon Ridge Research Station, Winnsboro, Louisiana in 2006. *Rhopalosiphum padi* was collected from winter wheat at the LSU AgCenter Sweet Potato Research Station (Chase) in Franklin parish, Louisiana in February 2012. The aphid colonies were established from single aptera and maintained under laboratory conditions in screened cages (30 x 30 x 30 cm, assembled using Plexiglass and nylon mesh fabric) at room temperature (20-22°C) and a 14L:10D photoperiod. *Aphis gossypii* was reared on cotton plants (*Gossypium* spp) cv. Stoneville 474, *M. persicae* was reared on mustard plants (*Brassica cretica* L.) cv. Tendergreen, while *R. padi* was reared on wheat plants (*Triticum aestivum*) cv. LA 841. The rearing plants have not been described as a host for sweetpotato viruses. Cotton and mustard plants were grown in the greenhouse under wide temperature (10-32°C) and humidity (21-98%) ranges, were fertilized on a weekly basis with NPK 20-20-20 (Scotts-Sierra Horticultural Products Company) and kept free of insecticides. Wheat plants were grown

in a growth chamber with temperature set at 25°C, 14L:10D photoperiod and humidity at 80%. A cohort of 5 to 10 aphids was placed on fresh plants using a paint brush to establish a new colony every 2 to 3 weeks.

5.2.4 Establishment of virus-infected host plants

Mixed virus-infected sweetpotato cvs. Beauregard and Evangeline plants were established using single node cuttings from plants that were previously graft inoculated with the naturally infected sweetpotato cv. Beauregard (B 14, G-7). Virus tested sweetpotato cv. Beauregard was graft inoculated with SPFMV-RC isolate 95-2 using scions from infected SOH plants. The isolate was maintained in SOH by serial mechanical inoculations and routinely tested for SPFMV by NCM-ELISA. Two wedge grafts were made per plant by inserting a single-node vine segment from the source plant into a slit in the stock plant. Only those on which scions survived for 3 weeks were used. Since titers in sweetpotato are often too low for detection by ELISA (Clark et al., 2012), plants were assayed for successful inoculation by grafting onto the standard virus indicator plant, Brazilian morning glory (*Ipomoea setosa*), and only those that produced typical SPFMV symptoms were used for study. *Ipomoea hederacea* and *I. cordatotriloba* seedlings were mechanically inoculated with SPFMV-RC, isolate 95-2. Carborundum-dusted cotyledons of plants were rubbed approximately 5 to 7 days after planting with sap extracts from *I. nil* plants in which the isolate was maintained. Sap was obtained by grinding small leaf portions expressing symptoms in 1 ml of inoculation buffer (0.05 M sodium phosphate with 0.01 M diethyldithiocarbamic acid [DIECA]) using a sterilized mortar and pestle. Plants were rinsed with distilled water after inoculation.

5.2.5 Colonization and survivorship studies

Ten plants of sweetpotato cv. Beauregard with mixed virus infection for each aphid species treatment were established in 10 cm diameter plastic pots containing autoclaved soil mix consisting of 1 part river silt, 1 part sand, 1 part Jiffy-Mix Plus (Jiffy Products of America Inc., Norwalk, OH) and 3.5 g/pot Osmocote 14-14-14 (Scotts-Sierra Horticultural Products Company). The plants were used for studies four weeks after cuttings were planted. Adult apterous aphids were obtained from the respective colonies and transferred into 1.5 ml vials using a camel's hair brush. Two apterous adults were placed separately on the abaxial surfaces of the second and third unfolded leaves from the top of each individual plant using 1cm diameter x 1 cm height clip cages (Davis et al., 2008) and allowed to larviposit for 24 hours. After nymphs were deposited, the adult and all but a single first instar were removed from each cage (Davis et al., 2008). The aphids were monitored for survival on a daily basis until they matured and began reproducing (Davis et al., 2008). The reproducing aphids were transferred to the nearest fresh leaf after every two to three days to limit effects of deteriorating leaf quality. This experiment was carried out under laboratory conditions (room temperature 20-22°C, and a 14L:10D photoperiod). The experiment was repeated three times with 20 aphids per replicate.

5.2.6 Life table studies for *Myzus persicae*

Colonizing aphids are those aphid species that settle and reproduce on the host in question. Twenty plants of each plant species/cultivar for each treatment were established in 10 cm diameter plastic pots containing autoclaved soil mix consisting of 1 part river silt, 1 part sand, 1 part Jiffy-Mix Plus (Jiffy Products of America Inc.) and 3.5 g/pot Osmocote 14-14-14 (Scotts-Sierra Horticultural Products Company). The non-

infected and the virus-infected plants were used for life table studies three weeks after virus inoculation. Adult apterous aphids were obtained from the colony and transferred into 1.5 ml vials using a camel's hair brush, they were placed on plants and monitored as described above (colonizing and survivorship studies). Life tables were developed for each test plant following the methods of Birch (1948). Age (x), age-specific survival (l_x), days to reproductive adult (DTA), and number of progeny per female per day (m_x), and age-specific fecundity ($l_x m_x$) were calculated. Intrinsic rate of increase (r_m) was calculated by means of the following equations:

$$\sum e^{-r_m x} l_x m_x = 1 \quad \text{equation [1]}$$

Net reproductive rate (R_0) was calculated as:

$$\sum l_x m_x \quad \text{equation [2]}$$

Finite rate of increase (λ) was calculated as:

$$e^{r_m} \quad \text{equation [3]}$$

Doubling time (DT) was calculated as:

$$\frac{\ln(2)}{r_m} \quad \text{equation [4]}$$

Each experiment was repeated three times with 20 plants per replicate. Tests on non-infected and virus infected plants were carried out at the same time on each host. Greenhouse temperatures during the study ranged between 22.4 °C to 32.1 °C with an average of 24.6 °C. These temperatures are within the reported range for reproduction of *M. persicae* under fluctuating regimes (Davis et al., 2006).

5.2.7 Data analysis.

Intrinsic rate of increase (r_m) values were calculated using the Jackknife procedure described in Meyer et al. (1986). Analysis of Variance (ANOVA) was used for analysis of differences in intrinsic rate of increase, mean generation time, net reproductive rate, doubling time, and finite rate of increase using PROC GLM. Tukey's multiple range test was used to separate means, $P = 0.05$.

5.3 Results

5.3.1 Colonization and survivorship studies

Aphis gossypii apterae failed to deposit any progeny, and *R. padi* deposited only one nymph which perished within 24 hours. All the 60 apterae of *M. persicae* deposited progeny which survived on mixed virus-infected sweetpotato cv. Beauregard. *Myzus persicae* displayed a type II survivorship curve with essentially constant aphid death rates and 50% of the single nymphs left on leaves died after 14 days (data not shown). The mean pre-reproductive period duration of *M. persicae* on sweetpotato was 6.6 days and the mean number of progeny produced was 20 per female aptera.

5.3.2 Life table studies for *Myzus persicae*

Myzus persicae was able to reproduce on sweetpotato cvs. Beauregard and Evangeline, and the two morning glory species *I. cordatotriloba* and *I. hederacea*. The intrinsic rate of increase (r_m) was significantly greater on mixed virus-infected compared with SPFMV-infected or non-infected plants for sweetpotato cvs. Beauregard ($F = 41.71$; $df = 2, 168$; $P < 0.0001$) and Evangeline ($F = 27.42$; $df = 1, 118$; $P < 0.0001$) (Table 5.1 and 5.2). However, the r_m levels were significantly higher on non-infected compared

with SPFMV-infected *I. cordatotriloba* ($F = 42.97$; $df = 1, 82$; $P < 0.0001$) and *I. hederacea* ($F = 27.56$; $df = 1, 118$; $P < 0.0001$) (Table 5.3 and 5.4).

Mean generation time (T), was significantly shorter on virus-infected compared with non-infected sweetpotato cvs. Beauregard ($F = 7.55$; $df = 2, 168$; $P = 0.007$) and Evangeline ($F = 12.11$; $df = 1, 118$; $P = 0.006$) (Table 5.1 and 5.2), but it was significantly longer on virus-infected compared with non-infected *I. cordatotriloba* ($F = 27.53$; $df = 1, 82$; $P < 0.0001$) and *I. hederacea* ($F = 5.41$; $df = 2, 118$; $P = 0.0217$) (Table 5.3 and 5.4). The net reproductive rate (R_0), was significantly greater on virus-infected compared with non-infected sweetpotato cvs. Beauregard ($F = 10.67$; $df = 2, 168$; $P < 0.0001$) and Evangeline ($F = 4.04$; $df = 1, 118$; $P = 0.0466$) (Table 5.1 and 5.2), but it was significantly lower on virus-infected compared with non-infected *I. cordatotriloba* ($F = 54.17$; $df = 1, 82$; $P < 0.0001$) and *I. hederacea* ($F = 57.12$; $df = 1, 118$; $P < 0.0001$) (Table 5.3 and 5.4). The doubling time (DT), was significantly shorter on mixed virus-infected compared with non-infected sweetpotato cvs. Beauregard ($F = 36.47$; $df = 2, 168$; $P < 0.0001$) and Evangeline ($F = 30.08$; $df = 1, 118$; $P < 0.0001$) (Table 5.1 and 5.2), but it was significantly longer on virus-infected compared with non-infected *I. cordatotriloba* ($F = 217.77$; $df = 1, 82$; $P < 0.0001$) and *I. hederacea* ($F = 32.68$; $df = 1, 118$; $P < 0.0001$) (Table 5.3 and 5.4). The finite rate of increase (λ) was significantly greater on mixed virus-infected compared with non-infected sweetpotato cvs. Beauregard ($F = 42.68$; $df = 2, 168$; $P < 0.0001$) and Evangeline ($F = 27.12$; $df = 1, 118$; $P < 0.0001$) (Table 5.1 and 5.2), but it was significantly lower on virus-infected compared with non-infected *I. cordatotriloba* ($F = 40.67$; $df = 1, 82$; $P < 0.0001$) and *I. hederacea* ($F = 26.93$; $df = 1, 118$; $P < 0.0001$) (Table 5.3 and 5.4).

Age-specific survivorships (l_x) for *M. persicae* did not vary for non-infected or virus-infected status within hosts. Non-infected and virus-infected sweetpotato cvs. Beauregard and Evangeline, and *I. hederacea* displayed a type II survivorship curve with essentially constant aphid death rates, while *I. cordatotriloba* displayed a hyperbolic death curve (Fig. 5.1). However, aphids survived longer on virus-infected and non-infected sweetpotato cv. Evangeline compared with the other hosts. Fifty percent of the aphids died after 14 to 15 days on Beauregard and *I. hederacea*, on Evangeline a similar percentage died after 18 to 20 days into reproduction, while on *I. cordatotriloba* 50% of the aphids died after 2 to 4 days into reproduction.

Table 5.1. Life table statistics of *Myzus persicae* on non-infected vs. virus-infected sweetpotato cv. Beauregard.

Parameter	Non-infected	SPFMV-infected	Mixed-infected	P value
Intrinsic rate of increase (r_m)	0.336 b	0.332 b	0.366 a	< 0.0001
Mean generation time (T)	9.0 a	8.8 b	8.7 b	0.0070
Net reproductive rate (R_o)	21.8 c	24.7 b	27.7 a	< 0.0001
Doubling time (DT)	2.1 a	2.1 a	1.9 b	< 0.0001
Finite rate of increase (λ)	1.4 b	1.4 b	1.5 a	< 0.0001

Means followed by same letters within rows are not significantly different ($P > 0.05$; Tukey's test).

Table 5.2. Life table statistics of *Myzus persicae* on non-infected vs. mixed virus-infected sweetpotato cv. Evangeline.

Parameter	Non-infected	Mixed-infected	P value
Intrinsic rate of increase (r_m)	0.225 b	0.248 a	< 0.0001
Mean generation time (T)	11.4 a	10.9 b	0.0060
Net reproductive rate (R_o)	16.2 b	18.8 a	0.0466
Doubling time (DT)	3.1 a	2.8 b	< 0.0001
Finite rate of increase (λ)	1.2 b	1.3 a	< 0.0001

Means followed by same letters within rows are not significantly different ($P > 0.05$; Tukey's test).

Table 5.3. Life table statistics of *Myzus persicae* on non-infected vs. SPFMV-infected *Ipomoea cordatotriloba*.

Parameter	Non-infected	SPFMV-infected	P value
Intrinsic rate of increase (r_m)	0.113 a	0.027 b	< 0.0001
Mean generation time (T)	11.0 b	12.7 a	< 0.0001
Net reproductive rate (R_o)	4.55 a	0.77 b	< 0.0001
Doubling time (DT)	8.5 b	30.5 a	< 0.0001
Finite rate of increase (λ)	1.1 a	1.0 b	< 0.0001

Means followed by same letters within rows are not significantly different ($P > 0.05$; Tukey's test).

Table 5.4. Life table statistics of *Myzus persicae* on non-infected vs. SPFMV-infected *Ipomoea hederacea*.

Parameter	Non-infected	SPFMV-infected	P value
Intrinsic rate of increase (r_m)	0.310 a	0.267 b	< 0.0001
Mean generation time (T)	10.0 b	10.6 a	0.0217
Net reproductive rate (R_o)	23.9 a	18.4 b	< 0.0001
Doubling time (DT)	2.3 b	2.7 a	< 0.0001
Finite rate of increase (λ)	1.4 a	1.3 b	< 0.0001

Means followed by same letters within rows are not significantly different ($P > 0.05$; Tukey's test).

5.4 Discussion

Aphid performance varies with plant hosts. These results show that *M. persicae* is capable of colonizing and utilizing sweetpotato cv. Beauregard because it larviposited and its progeny survived on this host. Clones of *A. gossypii* and *R. padi* used in this study cannot colonize sweetpotato cv. Beauregard due to their failure to larviposit on this host. Host plant selection by aphids involves responses to a variety of physical and chemical plant characteristics but is mostly affected by gustatory cues detected during stylet penetration of peripheral plant tissues (Powell et al., 2006). Initiation of nymph deposition by aphids is an indication of host acceptance. It is stimulated by chemical cues from secondary or primary metabolites that are encountered during brief stylet penetrations (Powell et al., 2006).

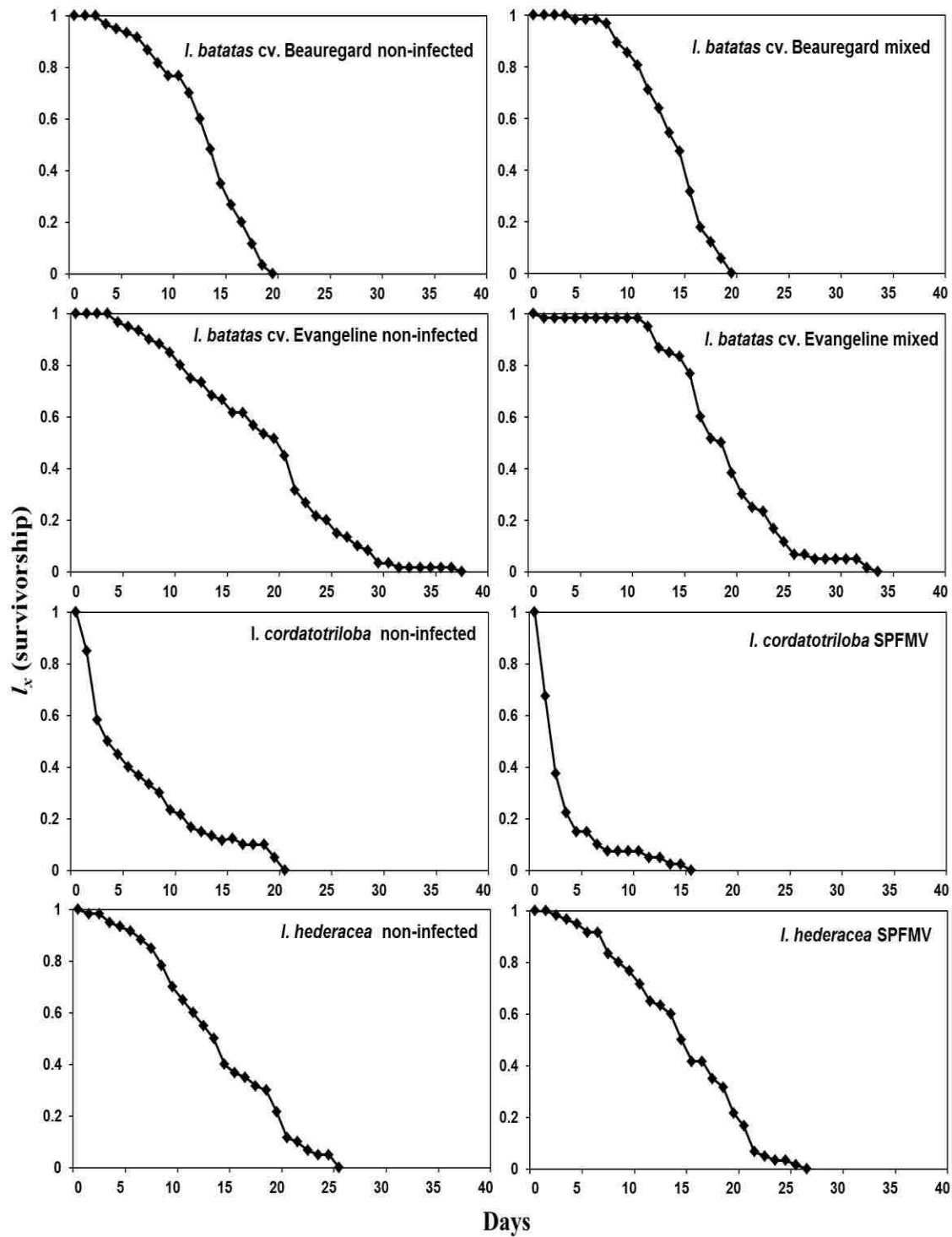


Fig. 5.1. Age-specific survivorships (I_x) for *Myzus persicae* on non-infected and virus-infected sweetpotato cvs. Beauregard and Evangeline, *I. cordatotriloba* and *I. hederacea*.

These metabolites may arrest aphids after landing on suitable hosts prompting them to settle and larviposit, but in a non-host they may deter aphid settling prompting them to initiate flight in search of a suitable host (Powell et al., 2006). These results suggest secondary metabolites possibly arrested *M. persicae* to settle, utilize and reproduce on sweetpotato cv. Beauregard, but they deterred *A. gossypii* and *R. padi* from utilizing this host. Although Blackman and Eastop (2006) have listed sweetpotato as a host of *A. gossypii*, our clone of this aphid species failed to survive and reproduce on sweetpotato cv. Beauregard. This may be due to differences in aphid clones and sweetpotato cultivars. Since *A. gossypii* and *R. padi* clones used in this study were collected from Louisiana, their failure to survive and reproduce on sweetpotato cv. Beauregard suggests that they probably do not utilize this host. The colonization status of *R. padi* on sweetpotato was un-known, and this is the first report indicating this aphid cannot utilize this host. *Myzus persicae* is a known efficient vector of sweetpotato potyviruses, and is frequent in Louisiana sweetpotato fields (Wosula et al., 2012). Its ability to utilize and reproduce on sweetpotato cv. Beauregard which is commonly grown in Louisiana suggests that aphid populations are likely to increase and this will enhance the possibility of sweetpotato potyvirus spread. *Aphis gossypii* is a very efficient vector of sweetpotato potyviruses (Souto et al., 2003; Wosula et al., 2012). Its inability to utilize sweetpotato suggests this crop will not contribute towards its population increase. However, the unsuitability of sweetpotato may prompt this aphid to probe and immediately depart in search of preferred hosts, and in the process, increase spread of sweetpotato potyviruses. A similar phenomenon will occur with *R. padi*, although it is a less efficient vector (Wosula et al., 2012). Aphids that wander around in search of suitable hosts spread non-persistent viruses more than those that settle and colonize their initial landing hosts (Racchah et al., 1985). *Myzus persicae*'s ability to colonize sweetpotato makes it a potential

vector of persistent viruses in this crop. Currently *Sweet potato leaf speckling virus* (SPLSV) a polerovirus is the only known persistent sweetpotato virus vectored by aphids, but it has not been found in the USA (Clark et al., 2012).

Virus-infected sweetpotato cvs. Beauregard and Evangeline were superior hosts for *M. persicae*, having the greatest reproduction rate based on all parameters measured compared with non-infected plants. The reproduction potential of *M. persicae* on sweetpotato cvs. Beauregard and Evangeline based on r_m (0.225 – 0.366) was comparable to what has been reported on other hosts considered suitable for this aphid (0.158 – 0.400) (Davis et al., 2006; Davis et al., 2007; Davis and Radcliffe, 2008; Fernández-Quintanilla et al., 2002; Nikolakakis et al., 2003; Sauge et al., 1998). Although the effects of viruses on physiological status of sweetpotato plants have not been evaluated, plants with mixed virus infection displayed mild virus symptoms (veinal chlorosis, yellow and purple chlorotic spots), while those infected only with SPFMV were asymptomatic. Growth rate, plant size and leaf size appeared similar for virus-infected and non-infected sweetpotato plants. Several authors have reported that virus-infected plants are superior for aphid reproduction compared to non-infected plants (Blua and Perring, 1992; Bosque-Pérez and Eigenbrode, 2011; Castle and Berger, 1993; Srinivasan et al., 2006). Increased performance on virus-infected plants has been attributed to increased amino acid and sugar concentrations in phloem sap (Ajayi, 1986; Blua et al., 1994; Fereres et al., 1990). This could have contributed to better performance of *M. persicae* on sweetpotato.

Virus-infected morning glories, *I. cordatotriloba* and *I. hederacea*, were inferior hosts to *M. persicae* compared with non-infected plants. The low r_m on SPFMV-infected *I. cordatotriloba* (0.027) was comparable to what has been reported on plant species considered to be poor hosts of *M. persicae* (Davis et al., 2008). Although the effects of viruses on

physiological status of morning glory plants have not been evaluated, virus infected *I. cordatotriloba* and *I. hederacea* had severe virus symptoms, and were stunted with reduced plant and leaf size, and leaf distortion compared with non-infected plants. Several authors have indicated virus-infected plants are inferior hosts to aphid reproduction compared with non-infected plants (Donaldson and Gratton, 2007; Hodge and Powell, 2008; Jiménez-Martínez and Bosque-Pérez, 2009; Mauck et al., 2010; Michels et al., 1994). Reduced performance on virus infected morning glories could be due to their poor growth and nutritional status due to reduced nutrient availability or activation of plant defense metabolites by the virus. Earlier studies indicated SPFMV titers are significantly greater in *I. hederacea* and *I. cordatotriloba* than sweetpotato cvs. Beauregard and Evangeline (Wosula et al., 2012). This is probably related to the severe disease symptoms which may reduce host quality status for *M. persicae*. Sap extracted for virus assays from *I. cordatotriloba* and *I. hederacea* plants showing severe virus symptoms discolors easily due oxidization compared with sap from non-infected plants due to increased phenolic compound production (Clark, C. A. personal communication). Phenolic compounds are known to deter herbivore feeding, and this could be a possible factor contributing to poor performance of *M. persicae* on virus infected morning glories (Howe and Jander, 2008). Virus infections may reduce photosynthetic capacity and nutrient availability within plants, and negatively affect aphid performance (Gao and Nassuth, 1993; Jiménez-Martínez and Bosque-Pérez, 2009). Williams (1995) suggested that host morphological changes such as leaf thickening due to virus infection may make it difficult for aphids to access the phloem. Herbers et al. (1997) found that distorted plasmodesmata occur within the phloem tissue of potato plants infected with *Potato leafroll virus* (PLRV), and that there was an altered carbohydrate allocation pattern causing impaired phloem sucrose loading, an accumulation of soluble sugars and starch,

and a reduced photosynthetic capacity of the leaves. Plants infected with viruses are also reported to produce increased quantities of salicylic acid for defense purposes (Thaler et al., 2010). Although salicylic acid is assumed to target pathogens, it can negatively affect performance of herbivores on virus infected plants. For example, induction of salicylic acid and proteinase inhibitors in tomato by *Tobacco mosaic virus* (TMV) reduced field colonization of tomato plants by *M. euphorbiae* and *M. persicae* (Thaler et al., 2010).

Survivorship of *M. persicae* on sweetpotato cvs. Beauregard and Evangeline, and *I. hederacea* displayed a type II survivorship curve which is associated with constant death rate. The shortest survival period was on sweetpotato cv. Beauregard, while the longest was on Evangeline. Survivorship of aphids varies depending on host plants (Srinivasan et al., 2008). The shorter survival period on Beauregard accompanied with high net reproductive rate indicate the ability of this host plant to enhance the clonal fitness of *M. persicae*. Aphids tend to concentrate their reproduction in early stages of reproductive life in order to maximize clonal fitness (Powell et al., 2006). The extended survivorship accompanied with low net reproductive rate on Evangeline could possibly lower the reproductive fitness of *M. persicae* and further expose it to parasites and predators (Davis et al., 2007).

Comparison of *M. persicae* performance among sweetpotato cultivars based on reproductive parameters indicates that sweetpotato cv. Evangeline is more resistant compared with Beauregard. The reduced performance of *M. persicae* on sweetpotato cv. Evangeline compared with Beauregard suggest that it could reduce aphid population pressure by increasing duration of development, survivorship and reducing fecundity, thus favoring population regulation by natural enemies (Davis et al., 2007). In a preliminary field study, (Davis, J. A. personal communication) with a large potato aphid (*Macrosiphum euphorbiae*) population,

populations were greater on Beauregard compared with Evangeline (up to 30 aphids/plant on Beauregard, and 19 aphids/plant on Evangeline).

This study indicates that the beneficial effect of viruses on the performance of *M. persicae* on sweetpotato could have implications on virus epidemiology and control strategy. According to McElhany et al. (1995) the complex result of the changing frequency of pathogen infected plants, local spatial structure of the host, and pathogen and vector populations determine the epidemiology of vector-borne pathogens. The increased reproductive fitness of *M. persicae* on virus infected sweetpotato cv. Beauregard based on high intrinsic rate of increase, reduced generation time, higher progeny numbers per reproductive female and shorter population doubling time suggest the aphid population is likely to increase rapidly hence aggravate spread of non-persistent viruses. Earlier studies (DiFonzo et al., 1997; Kantack et al., 1960; Wosula et al., 2012) have observed correlation between high aphid populations and spread of potyviruses in sweetpotato and potato fields. In addition our earlier study of aphid trapping revealed a high flight peak in 2009 at the St Landry field when *M. euphorbiae* heavily colonized sweetpotato plants in beds compared to 2010 and 2011 when no aphids were observed colonizing plants. Sweetpotato cvs. Beauregard and Evangeline are widely grown in Louisiana; the reduced *M. persicae* fitness on cv. Evangeline compared with cv. Beauregard suggests that growers can utilize this cultivar to minimize aphid population build up and virus spread. In a previous study (Wosula et al., 2012), *A. gossypii* and *M. persicae* failed to transmit SPFMV from mixed virus infected cv. Evangeline a phenomenon that was possibly due to low virus titer. Preliminary virus titer quantification from field samples indicates that cv. Evangeline has lower SPFMV virus titer compared with cv. Beauregard (Wosula, E. N. unpublished). These attributes in cv. Evangeline (reduced aphid reproduction and low virus titer) could be exploited in breeding

programs to minimize the spread of sweetpotato potyviruses. Sweetpotato cv. Beauregard infected with SPFMV alone does not seem to affect the fitness of *M. persicae* compared with non-infected plants, suggesting single infections will not impact the population dynamics of this aphid. Nonetheless, under field conditions a majority of the sweetpotato plants are always infected with two or more viruses (Clark et al., 2010).

The increase in population on mixed virus-infected sweetpotato cv. Beauregard will not only pose a threat to spread of potyviruses in sweetpotato but also viruses in other crops commonly grown in Louisiana, and in most cases found in close proximity with sweetpotato. For example *Soybean mosaic virus* (SMV) in soybean (*Glycine max*) is present in Louisiana soybean fields (Valverde, 2011), and *M. persicae* is one of its known efficient vectors (Halbert et al., 1981). This aphid species could also be responsible for vectoring *Sugarcane mosaic virus* and *Sorghum mosaic virus* in sugarcane (*Saccharum officinarum*), one of the most important crops in Louisiana (Grisham, 1994).

The negative effect of SFPMV-infected *I. cordatotriloba* and *I. hederacea* on *M. persicae* may prompt aphids to depart and search for healthy plants or better hosts after landing and probing virus infected plants of these species, and in the process enhance spread of sweetpotato potyviruses. These morning glories are known hosts of SPFMV in Louisiana (*I. cordatotriloba* occasionally acts as a perennial reservoir) (Clark et al., 1986), also tend to have high virus titers compared to sweetpotato (Wosula et al., 2012). The increased probability of *M. persicae* to depart from virus infected plants (due to poor nutritional status) which already have high virus titer will likely enhance the spread of non-persistent viruses. This behavior could also occur with other aphid vectors landing on these morning glories. Sweetpotato growers should control weedy morning glory plants in order to minimize their role in the spread of sweetpotato

potyviruses. The knowledge from this study can also be used to minimize the impact of sweet potato virus disease (SPVD) by reducing the availability of SPFMV inoculum. This can be achieved through use of cultural practices such as use of clean planting material, eliminating the old crop before establishing new crop, control of weeds known to be hosts of SPFMV within and around sweetpotato fields.

Eigenbrode et al (2002) reported that PLRV-infected potato attracts and arrests movement of *M. persicae*. This ability of virus infected plants to attract or arrest movement of aphids could minimize spread of non-persistent viruses. There is need to further study the olfactory behavioral responses of *M. persicae* to sweetpotato potyviruses in different hosts to determine if it will prefer virus infected sweetpotato compared with morning glories. There is need to also study the effect of viruses on the physiology of sweetpotato with regard to accumulation of amino acids and sugars which have been reported to increase under infection in other crops (Ajayi, 1986; Blua et al., 1994; Fereres et al., 1990).

CHAPTER 6: STYLET PENETRATION BEHAVIORS OF *MYZUS PERSICAE* ON FOUR *IPOMOEA* SPP. INFECTED OR NON-INFECTED WITH SWEETPOTATO POTYVIRUSES

6.1 Introduction

Sweetpotato [*Ipomoea batatas* (L.) Lam.], a member of the family *Convolvulaceae*, is an important food crop especially in Africa and parts of Asia where it is relied upon during famine (Loebenstein and Thottappilly, 2009). In the USA, sweetpotato demand is increasing given its perception as a nutritious food with more processed products becoming available (Clark et al., 2010). Sweetpotato is vegetatively propagated and is prone to accumulate viruses which cause cultivar yield decline and reduce storage root quality (Clark and Hoy, 2006; Clark et al., 2010).

In the USA, the most common viruses are the potyviruses: *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato virus G* (SPVG) and *Sweet potato virus 2* (SPV2, synonym = *Ipomoea vein mosaic virus*) (Clark et al., 2012). *Sweet potato virus C* (SPVC) a distinct species which was formerly SPFMV-common strain (Untiveros et al., 2010) may also be prevalent since it has been detected among field isolates that were previously obtained through natural aphid inoculation of sentinel plants (*Ipomoea setosa*), and detected and identified using a newly developed multiplex RT-PCR technique (Li et al., 2012). Sweetpotato potyviruses commonly occur as mixed infections in the field (Souto et al., 2003). Mixed infections of SPFMV, SPVG and SPV2 in sweetpotato cv. Beauregard usually show mild chlorotic spotting and veinal chlorosis or no symptoms, and cause yield losses of less than 15% (Clark et al., 2010). Sweetpotato potyviruses also infect other members of the morning glory family, many of which occur as wild plants or weeds in cultivated fields. In Louisiana the most common morning glory species within or around sweetpotato fields are *Ipomoea cordatotriloba* Dennst. (synonym *I.*

trichocarpa Ell., cotton morning glory) and *I. hederacea* Jacq. (ivy-leaf morning glory) (Clark et al., 1986).

Sweetpotato potyvirus are spread under field conditions by several aphid species (Stubbs and McLean, 1958; McLean, 1959; Loebenstein and Thottappilly, 2009) in a non-persistent manner (McLean, 1958; Kennedy et al., 1962). From past reports, aphids that transmit include *Aphis gossypii* Glover, *Aphis craccivora* Koch, *Myzus persicae* (Sulzer) and *Lipaphis pseudobrassicae* (Davis) (McLean, 1959; Loebenstein et al., 2009). In Louisiana the known efficient vectors are *A. gossypii* with transmission efficiency range of 0 to 39%, and *M. persicae* (0 to 22%) depending on virus host source and infection status. *Rhopalosiphum maidis* (Fitch) and *Rhopalosiphum padi* (L.) are inefficient vectors having efficiencies of 1% and 2% respectively (Wosula et al., 2012).

Sweetpotato potyviruses do affect the reproduction of *M. persicae* an efficient vector both positively and negatively depending on host plant. Sweetpotato cvs. Beauregard and Evangeline with mixed infections of potyviruses enhance the intrinsic rate of increase, but morning glory plants *I. cordatotriloba* and *I. hederacea* infected with SPFMV reduced the intrinsic rate of increase of *M. persicae* (Wosula, E. N. unpublished).

Probing and feeding behavior by aphids and other piercing and sucking insects can be monitored by means of electronic devices (Feres and Moreno, 2009). McLean and Kinsey (1964) developed the first equipment, but these techniques have been improved to give more precise and relevant information on the insect activities and position of the stylet inside the plant (Backus and Bennett, 1992; Tjallingii, 1988). Electronic devices based on DC-amplifiers commonly referred to as electrical penetration graph technique (EPG) can distinguish between the intercellular and intracellular environment, which makes it possible to know when plant cells

are punctured by insect stylets (Tjallingii, 1985). Electrical penetration graph techniques (Tjallingii, 1988) have been used widely to study host plant resistance to aphids (Davis and Radcliffe, 2008; Diaz-Montano et al., 2007; Montllor and Tjallingii, 1989; van Helden and Tjallingii, 2000) as well as the effect of several behavior modifying compounds (Nisbet et al., 1993; Powell, 1992). Different EPG waveforms have been characterized (A, B, C, pd, E1, E2, F, and G) and their correlations with the position of the stylet tips in the plant tissue (van Helden and Tjallingii, 2000). The waveforms reveal different insect activities, such as mechanical stylet work, salivation, sap ingestion, and position of the stylet tips within the plant (Tjallingii, 2006). The waveforms are grouped into three main behavioral phases: pathway phase, phloem or sieve element phase, and xylem phase (Tjallingii, 2006). The pathway phase (A, B, and C) constitutes multiple stylet penetration activities such as intercellular stylet insertion and withdrawal, periods of no stylet movement, and brief intracellular punctures by stylet tips also known as potential drops or pds (Prado and Tjallingii, 1994). The pathway phase is very important because during this phase the insect locates the sieve element (primary ingestion site) and accepts or rejects the host (Jiang and Walker, 2001). The sieve element phase begins with a salivation period (E1), followed by phloem sap ingestion with continuous salivation (E2) (Tjallingii, 2006). The xylem phase (G) is related to water intake by aphids replenish their water balance (Spiller et al., 1990).

Electrical penetration graph techniques have also been used to study non-persistent virus transmission (Collar et al., 1997). Brief punctures of the cell membrane (potential drops) are necessary for acquisition and inoculation of potyviruses (Powell, 1991). Potential drops (pds) are typically 3 to 15 seconds in duration and they have been divided into three distinct subphases that may occur during the pd: subphases II-1, II-2, and II-3 (Collar et al., 1997; Martín et al., 1997; Powell et al., 1995). Subphase II-3 represents ingestion of cytosolic fluid by the aphid and

acquisition of non-persistently transmitted viruses from source plants (Martín et al., 1997; Powell et al., 1995). The inoculation of non-persistently transmitted viruses to non-infected plants occurs during subphase II-1 (Martín et al., 1997). Powell (2005) demonstrated that salivation occurs during subphase II-1, which supports the hypothesis proposed by Martín et al. (1997) that salivation is the behavior associated with inoculation of non-persistently transmitted viruses.

Vector activity and behavior are important determinants of the rate and extent of epidemic virus development (Jeger et al., 2004). Changes in the attraction between the aphid vector and the infected plant and changes in the benefits obtained by the aphid from this relationship will certainly influence the probability of virus dispersal (Alvarez et al., 2007). Strategies for controlling plant viruses depend highly on the understanding of the virus-plant-vector interactions (Alvarez et al., 2007). Recent studies suggest transmission rate of SPFMV by the *M. persicae* is greater from morning glory species *I. cordatotriloba* and *I. hederacea* compared with sweetpotato cvs. Beauregard and Evangeline (Wosula et al., 2012). More studies on these host plants suggest the reproduction of *M. persicae* is greater on mixed virus-infected sweetpotato compared with non-infected, but lower on SPFMV-infected morning glory plants compared with non-infected (Wosula, E. N. unpublished). The main objective of this study was to characterize the stylet penetration behaviors of *M. persicae* on virus-infected and non-infected sweetpotato cvs. Beauregard and Evangeline, *I. cordatotriloba* and *I. hederacea* to determine if there are differences in behaviors associated with non-persistent virus transmission and host acceptance.

6.2 Materials and methods

6.2.1 Host plants

To ensure that plants were initially free of viruses, sweetpotato plants were derived from virus-tested mericlones maintained by nodal propagation in tissue culture at the LSU AgCenter Department of Plant Pathology and Crop Physiology and *Ipomoea cordatotriloba* and *I. hederacea*, were established from seeds harvested from single plants that were grown in the greenhouse. All plants were grown in the greenhouse under wide temperature (20-32°C) and humidity (21-98%) ranges, in 10 cm diameter clay pots containing autoclaved soil mix consisting of 1 part river silt, 1 part sand, 1 part Jiffy-Mix Plus (Jiffy Products of America Inc., Norwalk, OH) and 3.5 g/pot Osmocote 14-14-14 (Scotts-Sierra Horticultural Products Company). Plants were not sprayed with insecticide.

6.2.2 Virus inoculum

The russet crack strain of SPFMV (SPFMV-RC, isolate 95-2) was maintained in *I. nil* cv. Scarlet O'Hara (SOH) in the greenhouse by repeated mechanical inoculation, and a naturally mixed infected sweetpotato cv. Beauregard (B 14, G-7) that was grown in fields in North Carolina for seven years and provided by G. C. Yencho (Dept. Horticultural Sciences, North Carolina State University, Raleigh) was maintained by vegetative propagation. B 14, G-7 was tested using RT-PCR and qRT-PCR and after grafting on *I. setosa* using NCM-ELISA, and found to be infected with SPFMV, SPVG and SPV2, but tested negative for *Sweet potato mild mottle virus*, *Sweet potato latent virus*, *Sweet potato chlorotic fleck virus*, *Sweet potato mild speckling virus*, *Sweet potato leaf curl virus*, *Sweet potato chlorotic stunt virus*, *Sweet potato collusive virus*, and *Cucumber mosaic virus*. However, the possibility that it was infected by viruses not yet recognized in sweetpotato cannot be eliminated. Recently, with the separation of

the common strain of SPFMV into a distinct virus species *Sweet potato virus C* (SPVC) (Untiveros et al., 2010), B14, G-7 was tested using the newly developed multiplex RT-PCR technique (Li et al., 2012) and was found to be also infected with SPVC.

6.2.3 Aphid colony

Myzus persicae was collected from an unknown host in 2004. The aphid colony was established from single aptera and maintained under laboratory conditions in screened cages (30 x 30 x 30 cm, assembled using Plexiglass plastic sheet and nylon mesh fabric) at room temperature (20-22°C) and a 14L: 10D photoperiod on mustard (*Brassica cretica* L.) cv. Tendergreen, which has not been described as a sweetpotato virus host. Plants were grown in the greenhouse under wide temperature (10-32°C) and humidity (21-98%) ranges, were fertilized on a weekly basis with NPK 20-20-20 (Scotts-Sierra Horticultural Products Company) and kept free of insecticides. A cohort of 5 to 10 aphids was placed on fresh plants using a paint brush to establish a new colony every 2 to 3 weeks.

6.2.4 Establishment of virus-infected host plants

Mixed virus-infected sweetpotato cvs. Beauregard and Evangeline plants were established using single node cuttings from plants that were previously graft inoculated with the naturally infected sweetpotato cv. Beauregard (B 14, G-7). Virus tested sweetpotato cv. Beauregard was graft inoculated with SPFMV-RC isolate 95-2 using scions from infected SOH plants. The isolate was maintained in SOH by serial mechanical inoculations and routinely tested for SPFMV by NCM-ELISA. Two wedge grafts were made per plant by inserting a single-node vine segment from the source plant into a slit in the stock plant. Only those on which scions survived for 3 weeks were used. Since titers in sweetpotato are often too low for detection by ELISA (Clark et al., 2012), plants were assayed for successful inoculation by

grafting onto the standard virus indicator plant, Brazilian morning glory (*Ipomoea setosa*), and only those that produced typical SPFMV symptoms were used for study. *Ipomoea hederacea* and *I. cordatotriloba* seedlings were mechanically inoculated with SPFMV-RC, isolate 95-2. Carborundum-dusted cotyledons of plants were rubbed approximately 5 to 7 days after planting with sap extracts from *I. nil* plants in which the isolate was maintained. Sap was obtained by grinding small leaf portions expressing symptoms in 1 ml of inoculation buffer (0.05 M sodium phosphate with 0.01 M diethyldithiocarbamic acid [DIECA]) using a sterilized mortar and pestle. Plants were rinsed with distilled water after inoculation.

6.2.5 Electrical penetration graph studies (EPG)

Electrical penetration graph studies were on both non-infected and virus-infected sweetpotato cvs. Beauregard and Evangeline, and *I. cordatotriloba* and *I. hederacea*.

The EPG studies were carried out as described in Davis et al. (2008). The experiments were conducted in a Faraday cage using a Giga 4 DC EPG amplifier with one Giga Ohm input resistance and an AD conversion rate of 100 Hz (Wageningen Agricultural University, The Netherlands). A DAS-800 Digital Acquisition Card (Keithley Instruments, Inc., Cleveland, OH) converted analog signals into digital, which were visualized and recorded using WinDaq/Lite software (DATAQ Instruments, Inc., Akron, OH). Feeding behavior waveforms identifying specific aphid probing activities were distinguished using characteristics listed in Tjallingii and Hogen Esch (1993). Apterous adults of *M. persicae* were removed from mustard plants on which they were reared and used in feeding behavior studies after a 20 to 30 minute fasting period. A 2 cm length of 25 μ m gold wire (Good Fellow Metal Ltd., Cambridge, UK) was attached to the aphid dorsum with silver conductive paint (Pelco Colloidal Silver no. 16034, Ted Pella Inc., Redding, CA). One test plant was placed in the Faraday cage. Next, four aphids were

placed each on the adaxial side of each of four randomly selected leaves. The feeding behavior was recorded for 4 hours, giving sufficient time for the aphid to phloem feed. This was repeated with fresh test plants for each set of aphids to give a total of 32 aphids per treatment.

The following stylet penetration behaviors were evaluated: percentage of aphids that initiated probing, percentage aphids that initiated potential drops (pd) (intracellular cell punctures), percentage of aphids that initiated potential drops with archlets (pulses produced during potential drop subphase II-3; they are associated with acquisition of virus particles), percentage of aphids that initiated xylem ingestion (G), percentage of aphids that initiated phloem salivation (E1), percentage of aphids that initiated phloem ingestion (E2), time to 1st probe, duration of first probe, time 1st pd, pd duration, potential drop subphase durations (pd II-1, pd II-2 and pd II-3), average pds per probe, time to xylem ingestion, xylem duration, time to phloem ingestion, E1 phloem phase duration, E2 phloem phase, non-probing (np) duration (total/aphid), average probes per aphid, and average probes without potential drops. Intracellular stylet penetration behaviors (potential drop and its phases pd II-1, pd II-2 and pd II-3) were evaluated for 20 minutes, while other general behaviors were evaluated for 4 hours from the first placement of aphids on plants.

6.2.6 Data analysis

Data for stylet penetration behaviors that involved time duration and averages were log transformed [$\log_{10}(x + 0.01)$], and Analysis of Variance (ANOVA) was used for analysis of differences using PROC GLM. Tukey's multiple range test was used to separate means, $P = 0.05$. Data for stylet penetration behaviors that involved counts were tested for differences using Fisher's Exact Test using PROC FREQ.

6.3 Results

6.3.1 Percentage of aphids that initiated stylet penetration behaviors

The percentage of aphids that initiated probing and xylem ingestion were not significantly different on virus-infected compared with non-infected plants on all hosts. On sweetpotato cv. Beauregard, 46% of aphids significantly initiated E2 phloem phase (phloem ingestion) on virus-infected compared with 17% on non-infected plants ($P = 0.0239$). Thirty nine percent significantly initiated sE2 phloem phase (sustained phloem ingestion > 10 min) on virus-infected compared with 14% on non-infected plants ($P = 0.0379$). The percentage of aphids that initiated potential drops, potential drops with archlets and E1 phloem phase (phloem salivation) were not significantly different among virus-infected and non-infected sweetpotato cv. Beauregard. On sweetpotato cv. Evangeline a greater percentage of aphids initiated E1 phloem phase on non-infected (67%) compared with virus-infected plants (36%) plants ($P = 0.0345$). Also 75% of the aphids that probed significantly initiated potential drops on non-infected compared with virus infected (30%) plants ($P = 0.0039$). The percentage of aphids that initiated potential drops with archlets, E2 phloem phase and sE2 phloem phase were not significantly different. On *I. cordatotriloba*, there were no significant differences in percentage of aphids that initiated potential drops, potential drops with archlets and E1 phloem phase on virus-infected and non-infected plants, and also no aphid attained E2 phloem phase on this host. On *I. hederacea*, the percentage of aphids that initiated potential drops with archlets was significantly greater on non-infected (56%) compared with virus-infected (18%) plants ($P = 0.0151$). There were no significant differences in percentage of aphids that initiated potential drops, E1, E2 and sE2 phloem phases.

6.3.2 Stylet penetration behaviors on sweetpotato cv. Beauregard

On sweetpotato cv. Beauregard, the following aphid stylet penetration behaviors were significantly different between virus-infected and non-infected plants; the duration of 1st probe (F = 8.81; df = 1, 55; P = 0.0045), potential drop duration (F = 16.13; df = 1, 360; P = < 0.0001), pd subphase II-1 duration (F = 30.36; df = 1, 360; P = < 0.0001) and pd subphase II-3 duration (F = 16.80; df = 1, 360; P = < 0.0001), number of potential drops per probe (F = 46.09; df = 1, 460; P = < 0.0001), E1 phloem duration (F = 6.50; df = 1, 43; P = 0.0144), probes per aphid (F = 27.40; df = 1, 55; P = < 0.0001), and probes without potential drops (F = 7.00; df = 1, 36; P = 0.0120) (Table 6.1). Time to 1st probe, time to 1st, pd subphase II-2 duration, time to xylem ingestion, xylem duration, E2 phloem phase duration, and time to E1 phloem phase were not significantly different among virus-infected and non-infected plants. Total phloem ingestion duration (E2) for all aphids was numerically greater on mixed virus infected compared with non-infected plants (Table 6.1).

Table 6.1. Stylet penetration behaviors of *Myzus persicae* on non-infected vs. mixed virus-infected sweetpotato cv. Beauregard.

EPG parameter	Non-infected	Mixed virus-infected	P value
General phases ^b (min)			
Time to 1st probe	0.98 a	2.18 a	0.1465
1st probe duration	3.05 b	13.93 a	0.0045
Time to 1st pd	0.48 a	0.33 a	0.3315
Time to xylem ingestion	67.54 a	70.48 a	0.8978
Xylem duration	39.36 a	32.02 a	0.666
Time to E1 phloem phase	55.92 a	54.48 a	0.9245
E1 phloem duration	2.76 b	6.64 a	0.0144
E2 phloem duration	60.93 a	27.09 a	0.2316
Total E2 duration	483.78 a	683.51 a	0.1081
Non-probing duration	29.80 a	22.26 a	0.2321
No of pds/probe	2.9 b	7.0 a	<0.0001
No of probes/aphid	12.3 a	4.2 b	< 0.0001
No of probes without pds	3.2 a	1.5 b	0.012

Table 6.1. continued

Intracellular phases ^c (sec)			
Pd duration	4.15 b	4.68 a	<0.0001
Pd II-1 duration	1.19 b	1.46 a	<0.0001
Pd II-2 duration	1.71 a	1.64 a	0.1905
Pd II-3 duration	1.26 b	1.58 a	<0.0001

^aMeans followed by same letters within rows are not significantly different ($P > 0.05$; Tukey's test).

^bGeneral phases: E1 = phloem salivation; E2 = phloem ingestion (duration in minutes).

^cIntracellular phases: Pd = potential drop; Pd II-1 = 1st phase of pd; Pd II-2 = 2nd phase of pd; Pd II-3 = 3rd phase of pd (duration in seconds).

6.3.3 Stylet penetration behaviors on sweetpotato cv. Evangeline

Sweetpotato cv. Evangeline had significant differences with the following aphid stylet penetration behaviors on mixed virus-infected and non-infected plants; Time to 1st probe ($F = 4.72$; $df = 1, 56$; $P = 0.0341$), time to 1st potential drop ($F = 13.15$; $df = 1, 56$; $P = 0.0009$), pd duration ($F = 13.65$; $df = 1, 620$; $P = 0.0002$), pd II-1 duration ($F = 21.52$; $df = 1, 620$; $P = < 0.0001$), pd II-3 duration ($F = 47.83$; $df = 1, 620$; $P = < 0.0001$), time to xylem ingestion ($F = 6.81$; $df = 1, 30$; $P = 0.0140$) non-probing duration ($F = 9.18$; $df = 1, 56$; $P = 0.0037$) and number of probes without pds ($F = 10.85$; $df = 1, 45$; $P = 0.0019$) (Table 6.2). Time to 1st probe, pd II-2, potential drops per probe, xylem duration, time to E1 phloem phase, and phloem E1 and E2 durations were not significantly different among virus-infected and non-infected plants. Total phloem ingestion duration (E2) for all aphids was numerically greater on non-infected compared with mixed virus-infected plants (Table 6.2).

Table 6.2. Stylet penetration behaviors of *Myzus persicae* on non-infected vs. mixed virus-infected sweetpotato cv. Evangeline.

EPG parameter	Non-infected	Mixed virus-infected	<i>P</i> value
General phases ^b (min)			
Time to 1st probe	1.92 a	1.61 a	0.6892
1st probe duration	7.75 a	4.43 b	0.0341
Time to 1st pd	0.83 a	0.17 b	0.0009
Time to xylem ingestion	51.74 b	122.14 a	0.014
Xylem duration	46.86 a	39.35 a	0.8483
Time to E1 phloem phase	78.21 a	70.57 a	0.7171
E1 phloem duration	4.60 a	3.44 a	0.542
E2 phloem duration	20.08 a	12.56 a	0.6734
Total E2 duration	132.35 a	12.56 a	0.4398
Non-probing duration	22.74 b	44.45 a	0.0037
No of pds/probe	4.1 a	3.6 a	0.1941
No of probes/aphid	8.9 a	11.4 a	0.3025
No of probes without pds	2.3 b	4.8 b	0.0019
Intracellular phases ^c (sec)			
Pd duration	4.15 a	3.86 b	0.0002
Pd II-1 duration	1.05 b	1.24 a	<0. 0001
Pd II-2 duration	1.38 a	1.32 a	0.2559
Pd II-3 duration	1.72 a	1.30 b	<0.0001

^aMeans followed by same letters within rows are not significantly different ($P > 0.05$; Tukey's test).

^bGeneral phases: E1 = phloem salivation; E2 = phloem ingestion (duration in minutes).

^cIntracellular phases: Pd = potential drop; Pd II-1 = 1st phase of pd; Pd II-2 = 2nd phase of pd; Pd II-3 = 3rd phase of pd (duration in seconds).

6.3.4 Stylet penetration behaviors on *Ipomoea cordatotriloba*

On *I. cordatotriloba*, the following stylet penetration behaviors were significantly different on SPFMV-infected and non-infected plants; duration of 1st probe ($F = 6.63$; $df = 1, 56$; $P = 0.0059$), time to 1st potential drop ($F = 4.59$; $df = 1, 53$; $P = 0.0392$), pd II-1 ($F = 20.49$; $df = 1, 263$; $P = < 0.0001$) and pd II-2 ($F = 39.48$; $df = 1, 263$; $P = < 0.0001$), probes per aphid ($F = 5.21$; $df = 1, 56$; $P = 0.0264$), and probes without potential drops ($F = 4.45$; $df = 1, 56$; $P = 0.0405$) (Table 6.3). Time to 1st probe, pd and pd II-3 durations, potential drops per probe, time to xylem ingestion, xylem duration, time E1 phloem phase, E1 phloem duration and non-probing duration were not significantly different. No aphid was able to initiate E2 phloem phase on

SPFMV-infected plants. Total phloem ingestion duration (E2) for all aphids was numerically greater on non-infected compared with SPFMV-infected plants (Table 6.3).

Table 6.3. Stylet penetration behaviors of *Myzus persicae* on non-infected vs. SPFMV-infected *Ipomoea cordatotriloba*.

EPG parameter	Non-infected	SPFMV-infected	<i>P</i> value
General phases ^b (min)			
Time to 1st probe	2.07 a	2.03 a	0.9703
1st probe duration	4.71 a	1.50 b	0.0059
Time to 1st pd	0.24 b	0.72 a	0.0392
Time to xylem ingestion	127.01 a	92.76 a	0.3344
Xylem duration	23.58 a	41.24 a	0.1848
Time to E1 phloem phase	74.91 a	102.51 a	0.3692
E1 phloem duration	4.84 a	3.24 a	0.2656
E2 phloem duration	4.53 a	0.00 a	0.5000
Total E2 duration	83.66 a	0.00 a	0.5000
Non-probing duration	36.91 a	40.05 a	0.7225
No of pds/probe	3.5 a	3.4 a	0.8119
No of probes/aphid	10.7 b	16.2 a	0.0264
No of probes without pds	2.7 b	4.9 a	0.0405
Intracellular phases ^c (sec)			
Pd duration	3.98 a	3.92 a	0.6384
Pd II-1 duration	1.10 b	1.30 a	<0.0001
Pd II-2 duration	1.59 a	1.25 b	<0.0001
Pd II-3 duration	1.30 a	1.37 a	0.2032

^aMeans followed by same letters within rows are not significantly different ($P > 0.05$; Tukey's test).

^bGeneral phases: E1 = phloem salivation; E2 = phloem ingestion (duration in minutes).

^cIntracellular phases: Pd = potential drop; Pd II-1 = 1st phase of pd; Pd II-2 = 2nd phase of pd; Pd II-3 = 3rd phase of pd (duration in seconds).

6.3.5 Stylet penetration behaviors on *Ipomoea hederacea*

On *I. hederacea* only three stylet penetration behaviors were significantly different on SPFMV-infected compared with non-infected plants. They were average pd duration ($F = 23.25$; $df = 1, 256$; $P = < 0.0001$), pd II-2 duration ($F = 106.22$; $df = 1, 256$; $P = < 0.0001$) and time to xylem ingestion ($F = 5.64$; $df = 1, 54$; $P = 0.0288$) (Table 6.4). Time to 1st probe, pd II-1 and pd II-3 durations, 1st probe duration, probes per aphid, pds per probe, xylem duration, time to E1 phloem phase, E1 phloem duration, E2 phloem duration, non-probing duration, and probes

without pds were not significantly different among virus-infected and non-infected plants. Total phloem ingestion duration (E2) for all aphids was numerically greater on non-infected compared with SPFMV-infected plants (Table 6.4).

Table 6.4. Stylet penetration behaviors of *Myzus persicae* on non-infected vs. SPFMV-infected *Ipomoea hederacea*.

EPG parameter	Non-infected	SPFMV-infected	<i>P</i> value
General phases ^b (min)			
Time to 1st probe	2.90 a	1.41 a	0.0738
1st probe duration	5.05 a	5.28 a	0.9362
Time to 1st pd	0.36 a	0.58 a	0.3188
Time to xylem ingestion	32.79 b	146.17 a	0.0288
Xylem duration	47.60 a	35.11 a	0.434
Time to E1 phloem phase	71.34 a	96.24 a	0.247
E1 phloem duration	9.43 a	10.08 a	0.8839
E2 phloem duration	36.73 a	16.77 a	0.1474
Total E2 duration	625.10 a	198.66 a	0.3044
Non-probing duration	32.32 a	20.10 a	0.1686
No of pds/probe	4.5 a	3.9 a	0.1731
No of probes/aphid	11.0 a	9.5 a	0.6035
No of probes without pds	2.0 a	3.5 a	0.0738
Intracellular phases ^c (sec)			
Pd duration	4.50 a	3.72 b	< 0.0001
Pd II-1 duration	1.15 a	1.14 a	0.9358
Pd II-2 duration	1.78 a	1.20 b	<0.0001
Pd II-3 duration	1.57 a	1.39 a	0.0602

^aMeans followed by same letters within rows are not significantly different ($P > 0.05$; Tukey's test).

^bGeneral phases: E1 = phloem salivation; E2 = phloem ingestion (duration in minutes).

^cIntracellular phases: Pd = potential drop; Pd II-1 = 1st phase of pd; Pd II-2 = 2nd phase of pd; Pd II-3 = 3rd phase of pd (duration in seconds).

6.4 Discussion

The response of *M. persicae* to potyvirus infected plants was host specific. This plays an important role in the epidemiology of these viruses since their spread might be influenced by the behavior of aphids upon landing and probing. The percentage of aphids that initiated probing and those that attained xylem ingestion was not significantly different on virus-infected and non-infected plants of sweetpotato and morning glory. This shows that *M. persicae* will attempt to

probe these hosts and reach the xylem regardless of their virus infection status. According to Powell et al. (2006) aphids attempt to probe after landing on the plant surface as a way of determining host suitability irrespective of the plant status. The percentage of aphids that initiated probes with potential drops was significantly greater on non-infected compared to virus infected sweetpotato cv. Evangeline, while on the other hosts the number were not significantly different. This shows that possibly the aphids made more frequent attempts to reach the phloem due to increase in phagostimulants on non-infected compared with virus infected cv. Evangeline (Prado and Tjallingii, 1994).

The percentage of aphids that initiated potential drops with archlets (pulses that occur during subphase II-3 of the potential drop) was significantly greater on non-infected *I. hederacea*, but no significant differences were observed in other hosts. Possibly the virus altered the quality of sap in *I. hederacea* that deterred *M. persicae* from prolonged potential drops on virus infected compared to non-infected plants. The percentage of aphids that attained E2 phloem phase (phloem ingestion) and sustained phloem ingestion (phloem ingestion > 10 min) was significantly greater on mixed virus-infected compared with non-infected sweetpotato cv. Beauregard, but not on the rest of the hosts. This suggests virus infection enhanced the suitability of sweetpotato cv. Beauregard to *M. persicae* enabling more aphids to attain phloem ingestion which is associated with host acceptance (Gabryś and Tjallingii, 2002).

Although duration of various stylet penetration behaviors varied, time to 1st probe, duration in xylem ingestion, and time to E1 phloem phase (phloem salivation) were not significantly different on virus-infected and non-infected plants of all hosts. This shows that potyviruses possibly do not influence the ability of *M. persicae* to initiate probing, reach the phloem and the time it spends in the xylem on our study hosts. Aphids initiated potential drops

earlier on mixed virus-infected compared with non-infected sweetpotato cv. Evangeline, while on *I. cordatotriloba* this duration was longer on SPFMV-infected compared with non-infected plants. Shorter durations by aphids to initiation of potential drops may enhance acquisition of non-persistent viruses from hosts (Fernández-Calvino et al., 2006).

Myzus persicae had a significantly longer time to 1st probe on virus-infected compared with non-infected sweetpotato cv. Beauregard, while on *I. cordatotriloba* this duration was shorter on virus-infected compared with non-infected plants. Additionally, the number of probes/aphid was significantly greater on SPFMV-infected compared with non-infected *I. cordatotriloba*. Numerous and short probes are an indication that the aphid encountered some mechanisms that deterred it from reaching the phloem, while fewer and longer probes suggest absence of negative factors that would cause withdrawal of the stylets (Kordan et al., 2012). The numerous repeated probing on SPFMV-infected could have been due to a tethering effect (Kordan et al., 2012), possibly aphids could have departed had they been free to move (Powell et al., 1993). This suggests that *M. persicae* will possibly probe and depart from virus-infected *I. cordatotriloba* and is more likely to spread potyviruses as opposed to when it lands on virus-infected sweetpotato cv. Beauregard.

Stylet penetration behaviors associated with enhanced acquisition of non-persistent viruses such as longer potential drop (pd) durations and pd subphase II-3, and increased number of pds/probe were significantly greater on virus-infected compared with non-infected sweetpotato cv. Beauregard but not on other hosts. This suggests that the influence of potyviruses on this aphid behavior may depend on host species. Boquel et al. (2012) reported increased mean duration of potential drops and potential drop subphase II-3 by *M. persicae*, and increased number of potential drops by *Sitobion avenae* (Fitch) on *Potato virus Y* (PVY) infected

potato plants compared with non-infected plants. Increased potential drop duration, potential drop sub-phase II-3, increased number of potential drops per acquisition probe, increased number of probes, reduced duration to probe initiation and reduced probe duration are attributed to increased transmission of non-persistent viruses (Collar et al., 1997; Collar and Fereres, 1998; Powell, 1991; Symmes et al., 2008). Increased potential drops could also indicate *M. persicae* spend more time puncturing into cell tissues and sampling possibly due to presence of phagostimulants (Prado and Tjallingii, 1994).

The E1 phloem phase associated with phloem salivation was significantly longer on mixed virus-infected compared with non-infected sweetpotato cv. Beauregard, but not with other hosts. Phloem salivation is used by phloem feeding insects to suppress plant defense responses induced in sieve elements. It contains a variety of enzymes such as polyphenol oxidase and peroxidases associated with detoxification of plant allelochemicals (Pettersson et al., 2007). The increased E1 phloem phase on mixed virus-infected cv. Beauregard possibly enabled *M. persicae* to suppress plant defense responses in order to spend more time in E2 phloem phase (phloem ingestion). Although, the duration spent in E2 phloem phase (phloem ingestion) was not significantly different in virus-infected vs. non-infected plants among all hosts, the total phloem duration was numerically greater on virus-infected compared with non-infected sweetpotato cv. Beauregard, while no aphid reached E2 phloem phase on SPFMV-infected *I. cordatotriloba*.

Earlier studies (Wosula, E. N. unpublished) showed that *M. persicae* has a greater reproduction rate on mixed virus-infected sweetpotato cv. Beauregard compared with non-infected. Other authors have reported enhanced phloem ingestion by aphids on virus-infected compared with non-infected plants. For example, *M. persicae* had increased phloem ingestion on potato infected with PVY (Boquel et al., 2011; Boquel et al., 2012), *Potato leafroll virus*

(PLRV) (Alvarez et al., 2007). *Sitobion avenae* had increased phloem ingestion on PVY-infected potato and on wheat infected with *Barley yellow dwarf virus* (BYDV) (Boquel et al., 2011; Fereres et al., 1990). *Myzus persicae* has been reported to have increased fitness on potato plants infected with PVY and PLRV compared with non-infected plants (Castle and Berger, 1993; Srinivasan and Alvarez, 2007).

Plants infected with viruses are reported to show increased carbohydrates and amino acids in their leaves (Markkula and Laurema, 1964; Ajayi, 1986; Blua et al., 1994). Volatile compounds emitted from PLRV-infected potato plants are known to attract and increase settling of *M. persicae* on virus infected compared with non-infected plants (Eigenbrode et al., 2002; Srinivasan and Alvarez, 2007). The reduced phloem feeding and the inability of *M. persicae* to attain E2 phloem phase in SPFMV-infected *I. cordatotriloba* could be attributed to the effect of antifeedant compounds within phloem vessels or changes in physical properties of the plant, causing aphids to ingest more from the xylem compared with aphids on non-infected plants (Boquel et al., 2011; Boquel et al., 2012).

Our earlier study showed reproduction of *M. persicae* is lower on SPFMV-infected *I. cordatotriloba* compared with non-infected plants (Wosula, E. N. unpublished). In potato plants, PVY infection triggers various defense mechanisms among them phytohormones (Petrovič et al., 1997; Kovač et al., 2009) that are well known to interfere with aphid feeding (Slesak et al., 2001; Brunissen et al., 2009). A similar mechanism was possibly involved in response of *M. persicae* to SPFMV-infected *I. cordatotriloba*. Other authors have reported negative effects of viruses on reproduction of aphids for example bean infected with *Bean yellow mosaic virus* (BYMV) negatively affected settling and performance of *Acyrtosiphon pisum* (Harris) (Power, 1996). Wheat infected with *Wheat streak mosaic virus* (WSV) negatively affected reproduction of

Schizaphis graminum (Rondani) (Michels et al., 1994). Williams (1995) suggested that host morphological changes such as leaf thickening due to virus infection may make it difficult for aphids to access the phloem. Herbers et al. (1997) found that distorted plasmodesmata occur within the phloem tissue of potato plants infected with Potato leafroll virus (PLRV), and that there was an altered carbohydrate allocation pattern causing impaired phloem sucrose loading, accumulation of soluble sugars and starch, and a reduced photosynthetic capacity of the leaves.

Our results suggest that spread of sweetpotato viruses will vary depending on virus host. Stylet penetrations behaviors suggest that *M. persicae* is more likely to depart after landing and probing on virus-infected *I. cordatotriloba* compared with virus-infected sweetpotato cv. Beauregard. Virus titers of SPFMV have been found to be higher in *I. cordatotriloba* and *I. hederacea* and transmission by *M. persicae* is greater from these morning glories compared with sweetpotato cvs. Beauregard and Evangeline (Wosula et al., 2012). Inducing *M. persicae* to depart from a host with high virus titer (*I. cordatotriloba*) could enhance virus spread compared to a low virus titer host (sweetpotato cv. Beauregard) that is likely to reduce movement of *M. persicae*. In potato, infection with PVY attracts and promotes progeny development of the efficient vector *M. persicae*, but promotes interplant movement of the less efficient vector *M. euphorbiae* (Boquel et al., 2011). Growers should control morning glory weeds within and around sweetpotato fields to minimize their role in spread of viruses. The electrical penetration graph technique can be useful for preliminary studies that involve many species of aphids to determine their stylet penetration behaviors in relation to virus transmission.

CHAPTER 7: SUMMARY AND CONCLUSIONS

Sweetpotato is an important food crop in the world with an annual production of approximately 130 million tons. Sweetpotato is vegetatively propagated and is prone to accumulate viruses which cause cultivar yield decline and reduce storage root quality. The most common sweetpotato viruses in the USA are members of the family *Potyviridae* and the genus *Potyvirus*: *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato virus G* (SPVG), *Sweet potato virus 2* and probably the newly named *Sweet potato virus C* (SPVC) formerly SPFMV common strain. These viruses also infected weedy morning glories that are found within or around sweetpotato fields. These viruses are transmitted in the field by various aphid species with *Aphis gossypii* and *Myzus persicae* as the commonly known efficient vectors. In the USA use of virus tested propagation material is the commonly used strategy to minimize impact of viruses on yield and quality. In Louisiana, despite the efforts to provide growers with virus tested propagation material, potyviruses still account for yield losses of up to 15%. This had been attributed to the rapid re-infection of the virus tested material. There was need to understand the epidemiology of these potyviruses in order to devise other management strategies in addition to use of virus tested propagation material.

In this study we found that host plant species, virus infection status (single vs. mixed), virus titer, and aphid species affect the transmission rate of SPFMV. This virus was transmitted at greater rates by *A. gossypii* compared with *M. persicae* from morning glory plants (*I. cordatotriloba* and *I. hederacea*) which also had greater SPFMV titers compared with sweetpotato (cvs. Beauregard and Evangeline) which had low titers. SPFMV was also transmitted at greater rates from mixed virus-infected sources compared with sources infected with SPFMV alone.

Aphids were present in Louisiana sweetpotato fields throughout the growing period although their abundance and species diversity fluctuated during and among seasons. Sentinel plants (*Ipomoea setosa*) placed in fields to monitor virus spread were more frequently infected during the period of late June to August, primarily with SPFMV and less commonly with SPVG or SPV2. Virus titers of SPFMV in sweetpotato leaf samples were notably higher in late June to August while those of SPVG and SPV2 had peaks both early in the season and later during the crop cycle. Morning glory plants found within sweetpotato fields grew as annuals and remained free of virus symptoms early in the season but developed symptoms during the period of late June to mid-July. Our results indicate that increased virus spread to sentinel plants was limited to late June to August, despite the fact that sweetpotato plants are normally in beds and fields from March to October. This period coincided with lower aphid populations, but higher virus titer in sweetpotato plants and the spread of sweetpotato viruses into wild morning glories.

Sweetpotato cvs. Beauregard and Evangeline, and *I. hederacea* were determined to be suitable hosts for *M. persicae* based on intrinsic rate of increase, while *I. cordatotriloba* was a poor host. Infection of these host plants with potyviruses increased reproduction of *M. persicae* on sweetpotato but reduced reproduction on the morning glory species. The reproduction of *M. persicae* was also significantly greater on sweetpotato cv. Beauregard compared with Evangeline. *Aphis gossypii* (efficient vector) failed to deposit progeny on mixed virus-infected sweetpotato cv. Beauregard, while *R. padi* (less efficient vector) deposited a single nymph that died within 24 hours.

Electrical penetration graph technique studies showed that infection of sweetpotato and morning glory plants with potyviruses either positively or negatively influences the stylet penetration behaviors of *M. persicae*. More aphids attained phloem ingestion on mixed virus-

infected sweetpotato cv. Beauregard compared with non-infected plants, while on *I. cordatotriloba* no aphid attained phloem ingestion on SPFMV-infected plants.

In the USA, potyviruses have been managed through reducing virus inoculum by using limited generation seed that was initially virus free, thereby continually flushing out the diseased material. Despite this effort, sweetpotato plants in Louisiana fields are frequently re-infected at high rates with the predominant potyviruses. Results from this study can be used to design additional management strategies to reduce re-infection of virus tested material. These strategies may include limiting availability of primary and secondary inoculum by separating beds and fields, separating seed plots from commercial crop, separating foundation seed plots from any potential sources of inoculum (plants established using materials from previous generations) and control of weedy morning glories. Little can be done to control the aphids since most of them are transient migrants that originate from other crops and plants in the landscape and not sweetpotato. Life stable studies indicate virus infected sweetpotato increases the reproduction of the vector *M. persicae*. Colonizing aphids found on sweetpotato should be controlled to reduce vector population build up. Further, use of insecticides to control aphids has not reduced spread of non-persistent viruses because of the very short period required for their acquisition and inoculation. According to our observations, morning glory plants within sweetpotato fields are mostly annuals that become infected in late June to mid-July, suggesting that sweetpotato itself is the major source of primary inoculum. Since it is not feasible to control aphids, sweetpotato seed production from virus tested materials should be carried out in areas free of any potential inoculum sources, mainly sweetpotato plants from previous generations. Growers should be advised to strictly separate their virus-tested seed material from other sweetpotato fields that could act as sources of inoculum. They could also time planting of fields so that seed crops are

not near older generation plantings during the critical window of virus spread. Although morning glory plants may not be sources of primary inoculum, they could act as sources of secondary inoculum once infected, hence extending potential sources of virus inoculum. Growers should control weedy morning glories in order to minimize their role in the spread of sweetpotato potyviruses.

In summary this study shows that transmission and spread of sweetpotato potyviruses depend on availability of both aphid vectors and sufficient inoculum titers, virus host species source, aphid vector species and whether infected with one or more viruses. Sweetpotato potyviruses do influence the reproduction and behavior of aphid vectors depending on the host plant. The positive effect of these viruses may enhance rapid reproduction of vectors leading to increased populations that can aggravate virus spread. The negative effect on aphid behavior may enhance departure from unsuitable hosts in search of better hosts. This may enhance spread of non-persistent viruses especially if the unsuitable hosts are infected and happen to have higher virus titers. Use of virus tested propagation material has greatly boosted sweetpotato yield in the USA compared to other parts of the world. However despite this effort growers still incur yield losses due to viruses. Findings from this study should be used to devise other cultural practices that will minimize spread of viruses, in addition to use of virus tested propagation material. These may include eliminating or reducing any possible sources of inoculum in foundation seed plots, planting beds and fields. Other strategies such as use of mineral oils, barrier crops and resistant cultivars could also be evaluated for their potential to reduce virus spread.

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APPENDIX A: THRIP ABUNDANCE IN SWEETPOTATO FIELDS

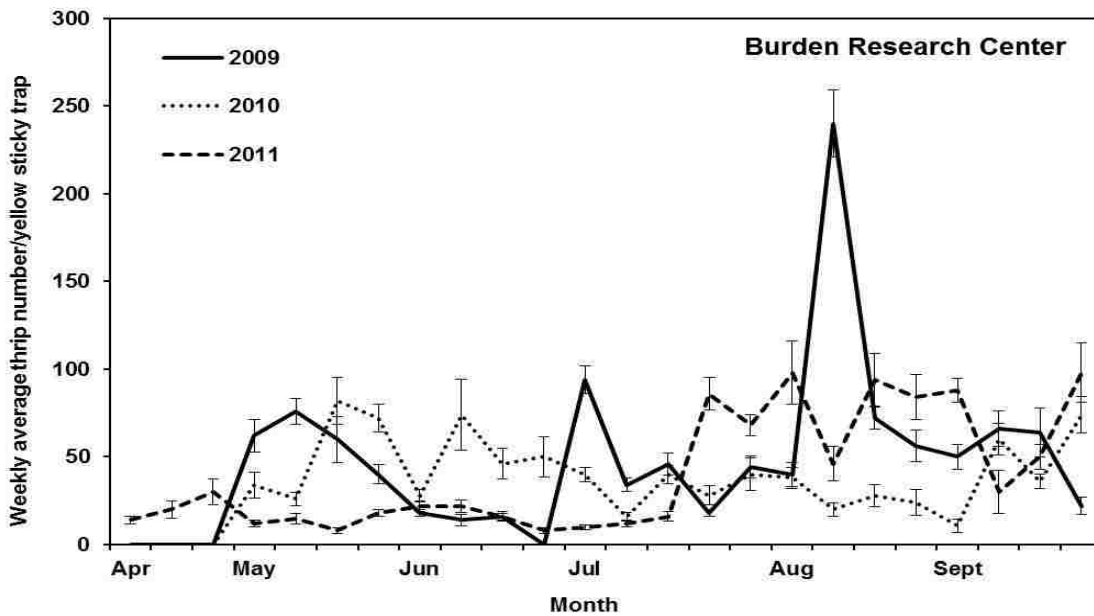


Fig. A.1. Weekly average number of thrips captured on yellow sticky traps (number/trap) in sweetpotato fields at the Burden Research Center in 2009 to 2011 (Means \pm SE).

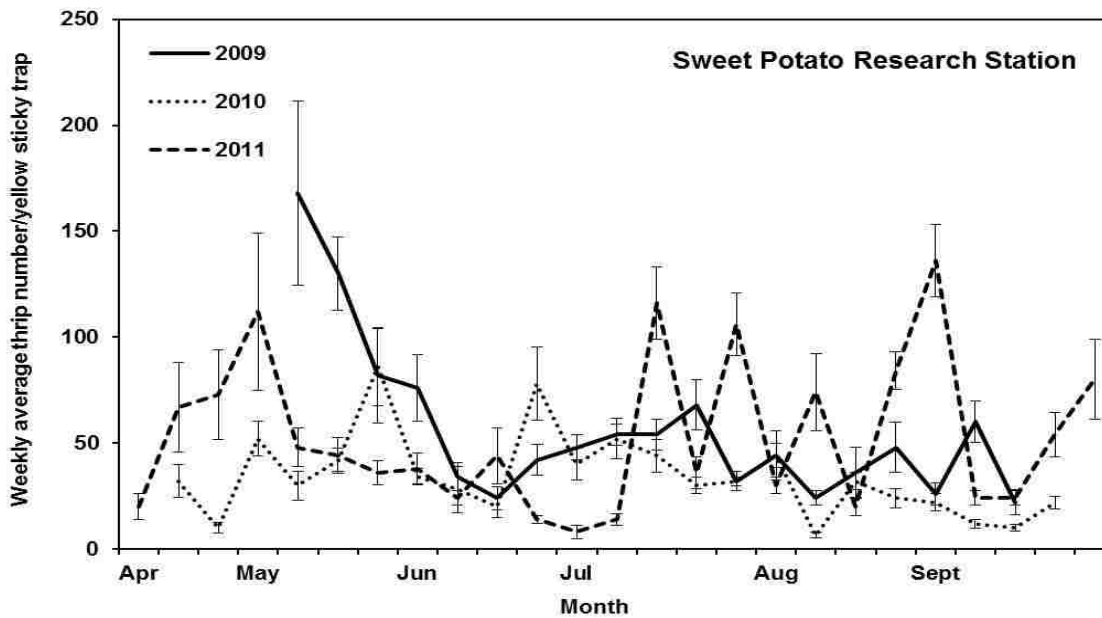


Fig. A.2. Weekly average number of thrips captured on yellow sticky traps (number/trap) in sweetpotato fields at the Sweet Potato Research Station in 2009 to 2011 (Means \pm SE).

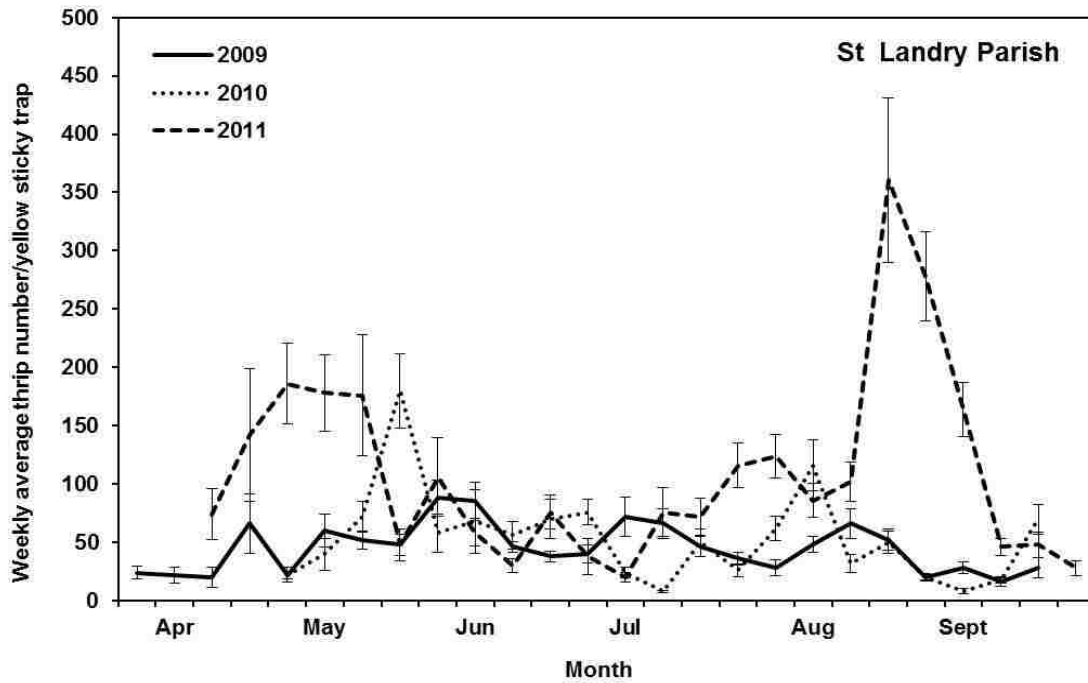


Fig. A.3. Weekly average number of thrips captured on yellow sticky traps (number/trap) in sweetpotato fields in St Landry parish in 2009 to 2011 (Means \pm SE).

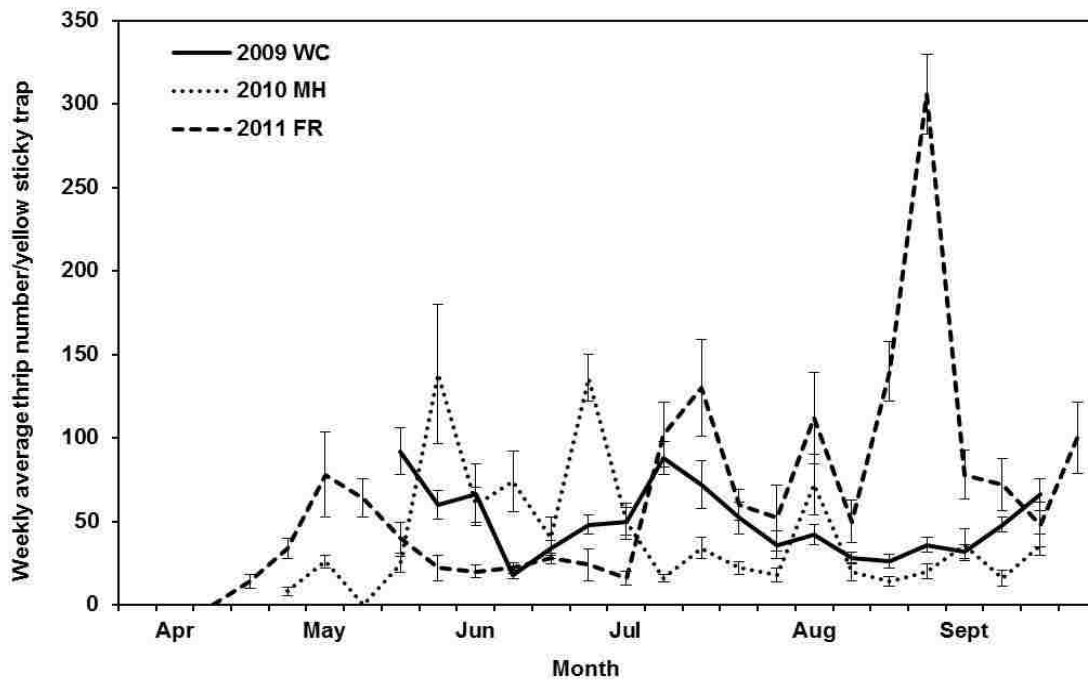


Fig. A.4. Weekly average number of thrips captured on yellow sticky traps (number/trap) in sweetpotato fields at West Carroll (WC), Morehouse (MH) and Franklin (FR) parishes in 2009 to 2011 (Means \pm SE).

APPENDIX B: WHITEFLY ABUNDANCE IN SWEETPOTATO FIELDS

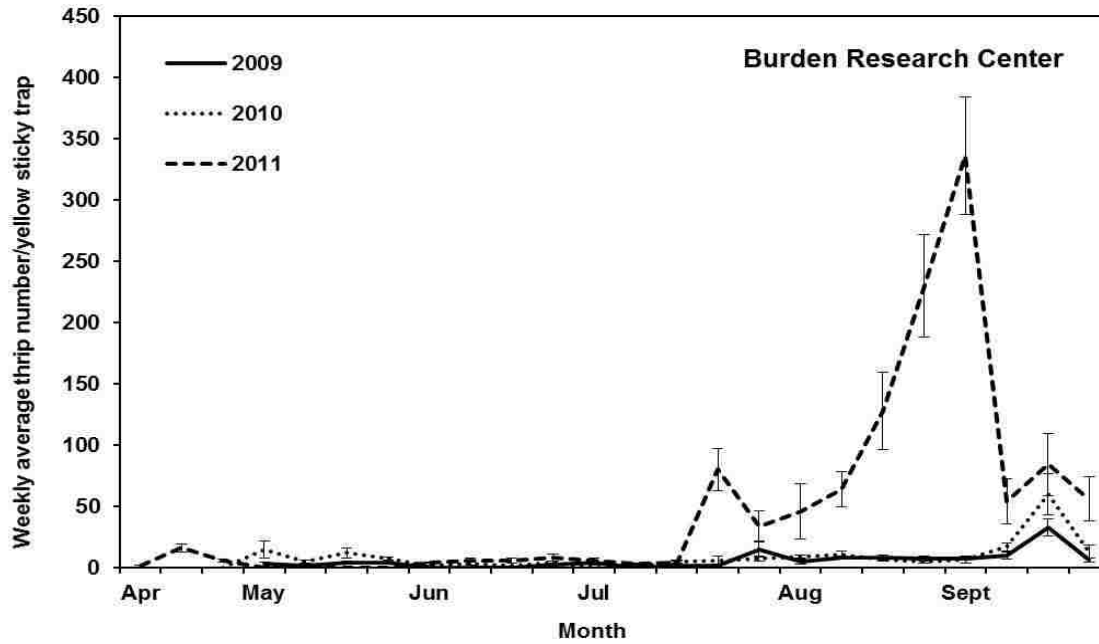


Fig. B.1. Weekly average number of whiteflies captured on yellow sticky traps (number/trap) in sweetpotato fields at Burden Research Center in 2009 to 2011 (Means \pm SE).

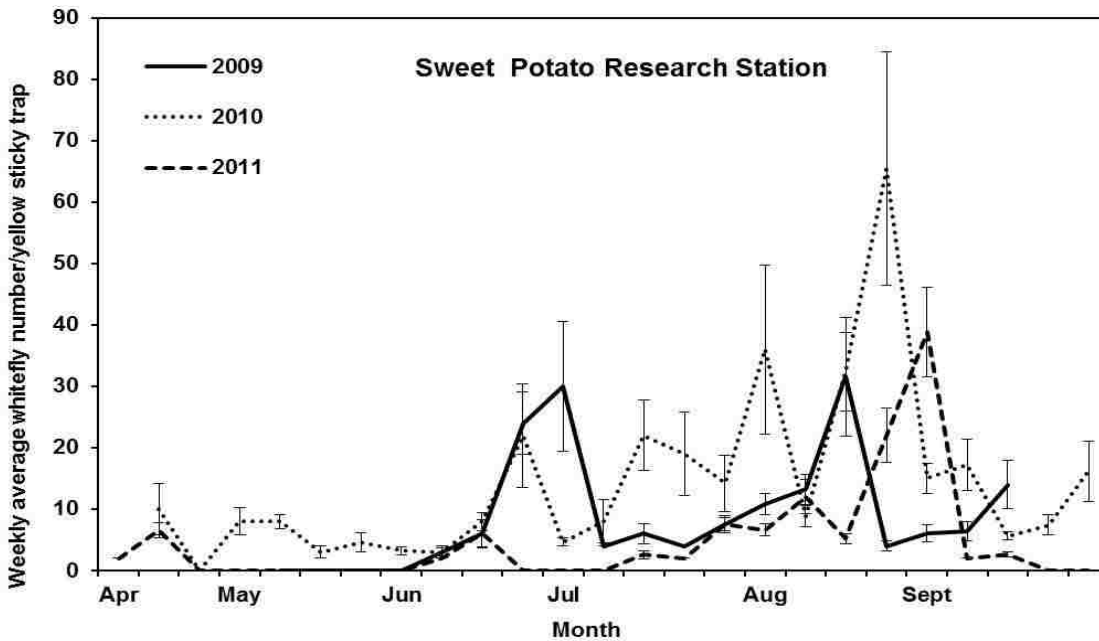


Fig. B.2. Weekly average number of whiteflies captured on yellow sticky traps (number/trap) in sweetpotato fields at Sweet Potato Research Station in 2009 to 2011 (Means \pm SE).

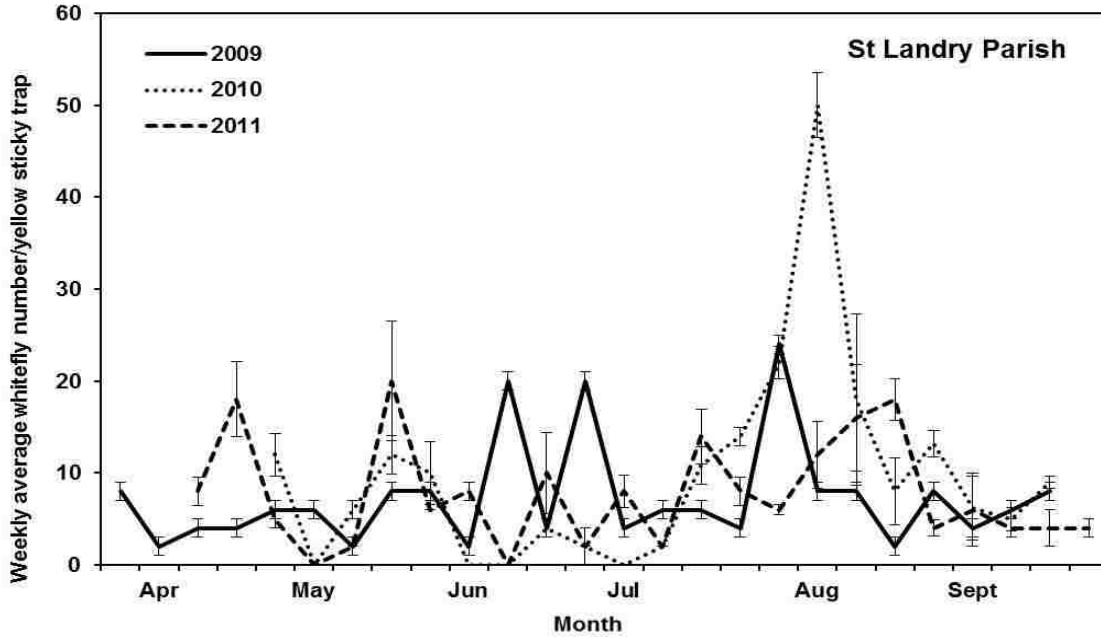


Fig. B.3. Weekly average number of whiteflies captured on yellow sticky traps (number/trap) in sweetpotato fields at St Landry parish in 2009 to 2011 (Means \pm SE).

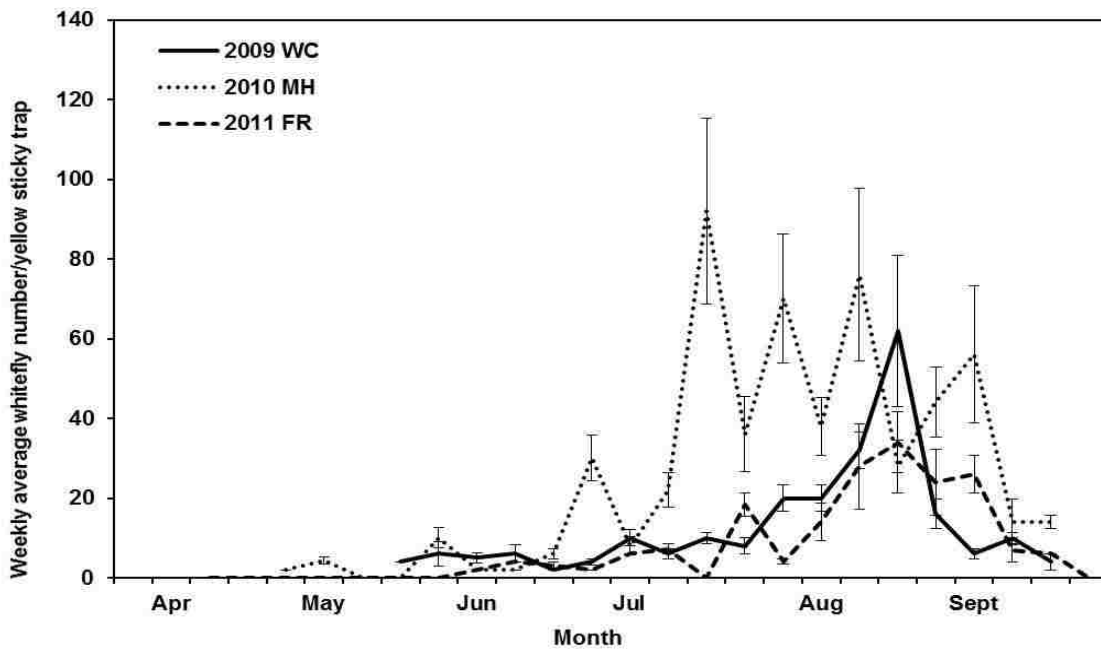


Fig. B.4. Weekly average number of whiteflies captured on yellow sticky traps (number/trap) in sweetpotato fields at West Carroll (WC), Morehouse (MH) and Franklin (FR) parishes in 2009 to 2011 (Means \pm SE).

APPENDIX C: ELECTRICAL PENETRATION GRAPH WAVEFORMS

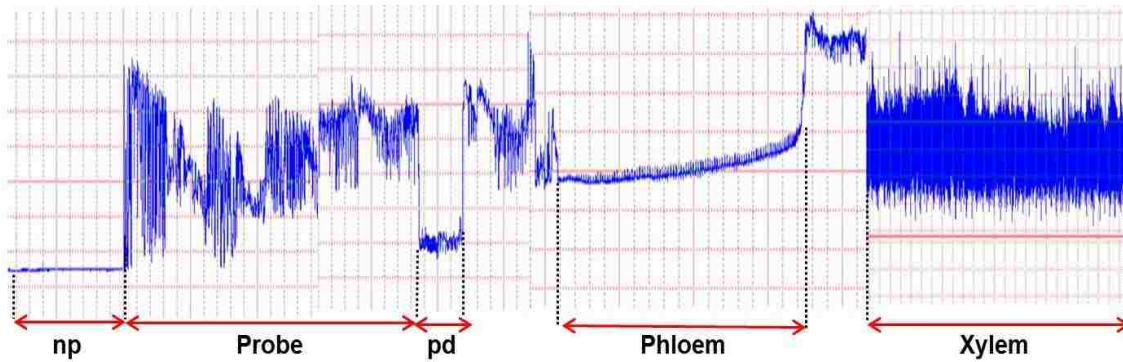


Fig. C.1. Aphid stylet penetration patterns: np = non probing (aphid not yet inserted stylet in the plant); probe (aphid stylet inside plant); pd = potential drop (stylet punctures plant cell); phloem (aphid salivates and ingests from phloem); xylem (aphid ingestion from the xylem).

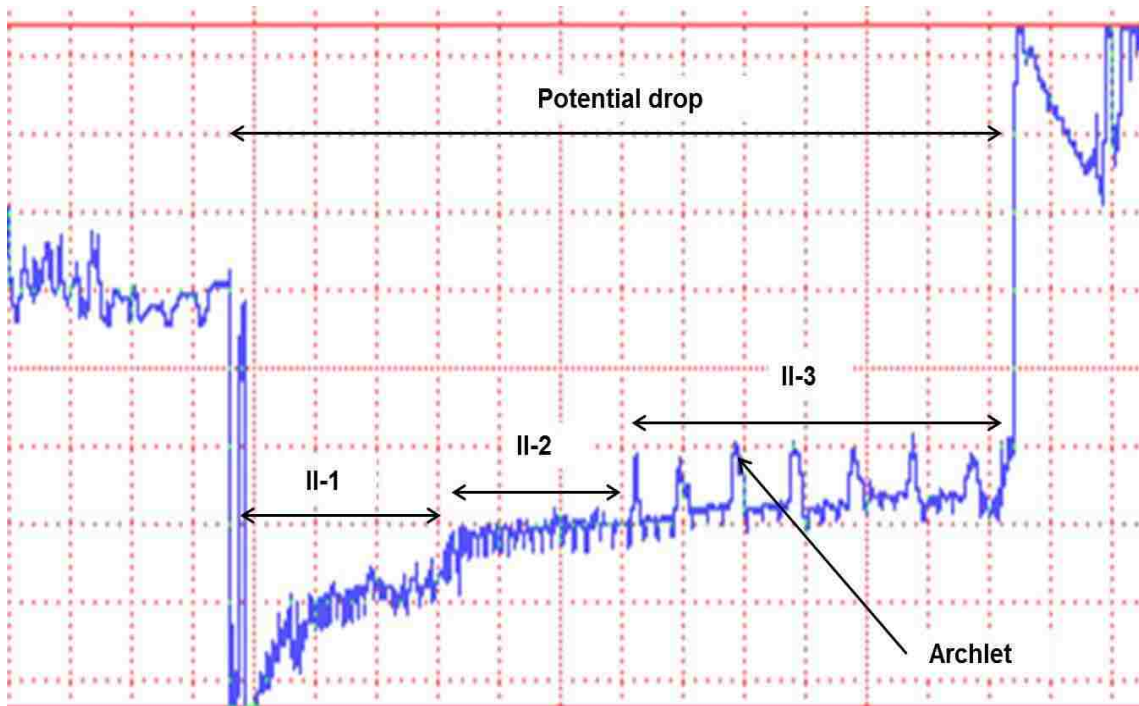


Fig. C.2. Potential drop (pd) subphases II-1 (aphid salivates); II-2 (unknown activity); III-3 (aphid ingests).

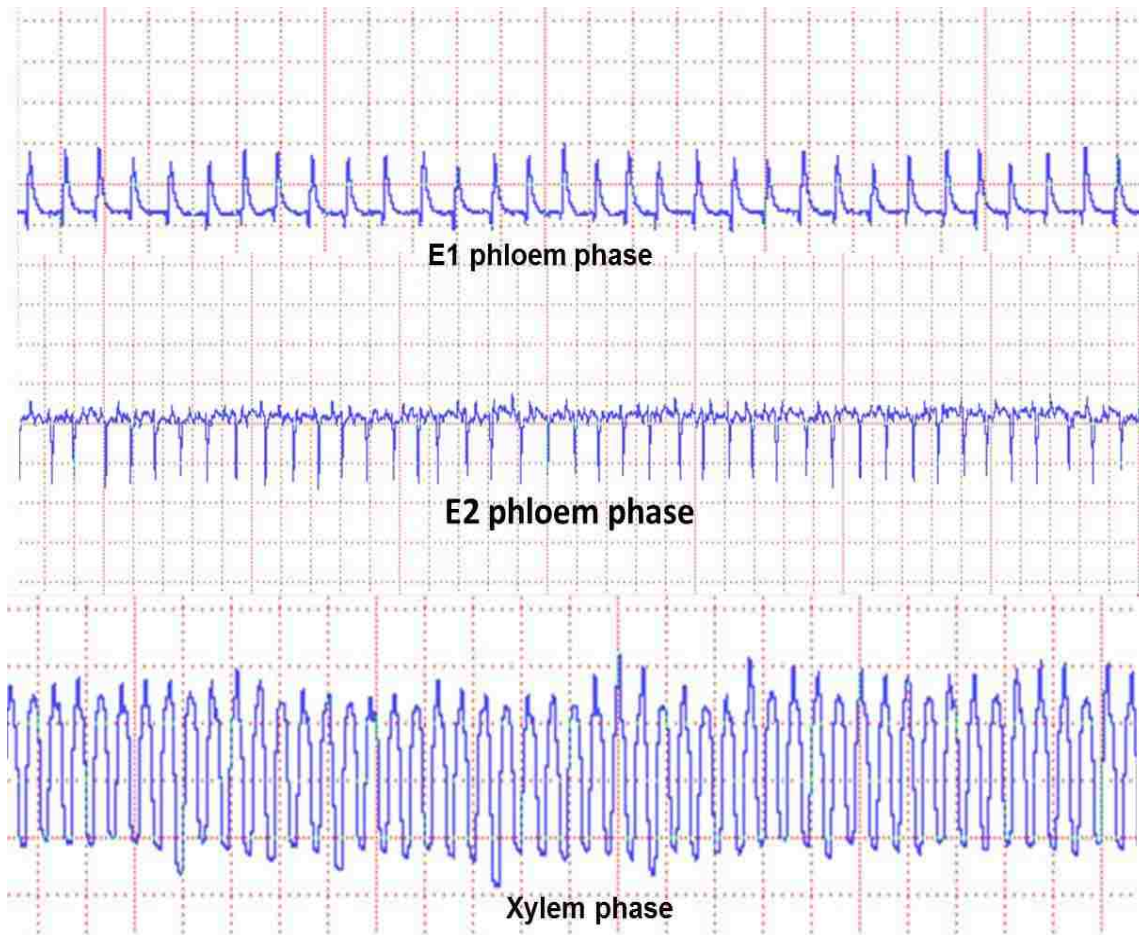


Fig. C.3. Wave form patterns of aphid stylet insertion into the phloem and xylem: E1 phloem phase (aphid salivates); E2 phloem phase (aphid ingests); Xylem phase (aphid ingests).

APPENDIX D: PERMISSION LETTERS

From: Everlyne Wosula [mailto:ewosul1@tigers.lsu.edu]
Sent: Wednesday, September 12, 2012 12:05 PM
To: Karen Cummings
Subject: Permission letters

Dear Dr Cummings,

I would like to request for permission letters to include all or part of the work that I have already published in Plant Disease into my doctoral dissertation.

My publications are:

Wosula, E. N., Clark, C. A., and Davis, J. A. 2012. Effect of host plant, aphid species, and virus infection status on transmission of Sweetpotato feathery mottle virus. Plant Dis. 96:1331-1336.

Wosula, E. N., Davis, J. A., Clark, C. A., Smith, T. P., Arancibia, R. A., Musser, F. R., and J. T. Reed. 2012. The role of aphid abundance, species diversity and virus titer in the spread of sweetpotato potyviruses in Louisiana and Mississippi. Plant Dis. <http://dx.doi.org/10.1094/PDIS-06-12-0564-RE>

Thank you,
Everlyne Wosula

From: Karen Cummings <kcummings@scisoc.org>
Date: Wed, Sep 12, 2012 at 2:26 PM
Subject: RE: Permission letters
To: Everlyne Wosula <ewosul1@tigers.lsu.edu>

Dear Ms. Wosula,

Thank you for your request to include the two articles from Plant Disease cited below in your dissertation. We are pleased to have you do so. Permission is hereby granted for you to reproduce the articles, all or in part, limited to your dissertation and provided the source is properly credited.

Good luck with your dissertation.

Best regards,

Karen Cummings

Director of Publications

The American Phytopathological Society

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St. Paul, MN 55121

phone: 651/994-3861

fax: 651/454-0766

VITA

Everlyne Nafula Wosula was born in 1974, in Bungoma County of the Republic of Kenya. After completing high school, she joined Jomo Kenyatta University of Agriculture and Technology and graduated in 1998 with a Bachelor of Science degree in Horticulture. After graduating, she started her career as a technical extension assistant with Kenya Tea Development Agency where she worked until 2002. She also worked as a field officer with Cereal Growers Association from 2003 to May 2004. In June 2004 to 2007, she was offered a joint scholarship by the International Center of Insect Physiology and Ecology (ICIPE) and Jomo Kenyatta University of Agriculture to pursue her Master of Science degree in Horticulture. She also worked as a research assistant in the Red spider mite and Cowpea projects at ICIPE in 2004 to 2007 under the supervision of Dr. Markus Knapp and Dr. Remy Pasquet. She joined the Sweetpotato pathology lab in the Department of Plant Pathology and Crop Physiology at Louisiana State University to pursue a doctoral degree in Fall 2008 under the supervision of Dr. Christopher A. Clark and Dr. Jeffrey A. Davis. She will receive the Degree of Doctor of Philosophy during the Fall Commencement 2012.