Effect of Host Plant, Aphid Species, and Virus Infection Status on Transmission of Sweetpotato feathery mottle virus

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Abstract

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Sweetpotato feathery mottle virus (SPFMV) is a nonpersistently transmitted virus known to infect sweetpotato (*Ipomoea batatas*) and wild morning glory plants. SPFMV is vectored by various aphid species, among them the green peach aphid, *Myzus persicae*, and the cotton aphid, *Aphis gossypii*. Our objective was to determine whether differences in acquisition hosts (sweetpotato and morning glory), aphid species (*M. persicae* and *A. gossypii*), and infection status (single versus mixed infection) influenced transmission of SPFMV. SPFMV transmission from *I. hederacea* with a natural mixed infection by *A. gossypii* (39%) was significantly greater than in other host-virus combinations. Successful transmissions by *A. gossypii* were significantly

greater compared with *M. persicae* in all host-virus combinations. Virus titers in source leaves were significantly greater in single- and mixed-infected *I. hederacea* and single-infected *I. cordatotriloba* compared with other host-virus combinations. There was a significant positive correlation between virus titer and transmission by both aphid species. These results suggest that, under controlled conditions, SPFMV is more readily transmitted from infected morning glory plants than from sweetpotato. Additionally, mixed-infected plants are better virus sources for transmission than single-infected, and *A. gossypii* is a more efficient vector than *M. persicae* under laboratory conditions.

Sweetpotato (*Ipomoea batatas* L.) 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 (13). It is increasing in demand in the United States because it is increasingly regarded as uniquely 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 (3,10). Sweetpotato is vegetatively propagated and is prone to accumulate viruses which cause cultivar yield decline and reduce storage root quality (7,8). The most common sweetpotato viruses in the United States are members of the family *Potyviridae* and the genus *Potyvirus*: Sweet potato feathery mottle virus (SPFMV), Sweetpotato virus G (SPVG), and Sweetpotato virus 2 (SPV2, synonym = *Ipomoea vein mosaic virus*) (5,9,33). Sweetpotato *Potyvirus* spp. are restricted primarily to the family *Convolvulaceae*, transmitted by aphids, and occur commonly as mixed infections in the field (3,24,35).

SPFMV is the most common sweetpotato virus and occurs in all sweetpotato-growing areas, including the United States (9). Four strains were originally differentiated: russet crack (RC), common (C), ordinary (O), and East African (EA) (17,21). The RC and C strains are widely distributed, whereas EA seems to be most common in Africa, though it has also been reported in Peru, Spain, and Easter Island (14,21,29,40). 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 (39). Single infections of SPFMV or mixed infections with SPVG and SPV2 in

‘Beauregard’ sweetpotato usually show mild chlorotic spotting and veinal chlorosis or no symptoms, and cause little or no yield loss (10,20). However, naturally infected sweetpotato plants (i.e., grown in the field for several years) which test positive for these three *Potyvirus* spp. and negative for other known sweetpotato viruses, may display distinct symptoms accompanied with yield reduction (7,10), possibly indicating the presence of other unknown viruses.

Mixed virus infections can positively or negatively impact transmission rates and alter patterns of virus spread (30). Mixed infections could be better sources of inoculum for some viruses whereas, for others, transmission rates are greater from single infections (30). 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%) (10). Because SPVC was not recognized as a distinct species until this year, data are not available for its occurrence.

Experimental host range studies suggest that other wild virus hosts are potential sources of inoculum, (9) mainly morning glory plants in the genus *Ipomoea*, family *Convolvulaceae* (22,38). In Louisiana, the morning glory species *I. 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 (6).

SPFMV is transmitted by several aphid species (35) in a nonpersistent manner (17,18). Aphid vectors acquire and transmit *Potyvirus* spp. to susceptible plants without necessarily colonizing the infected plants due to their sap sampling/probing feeding habit (27). These aphids acquire the virus in as short as 3 to 35 s (23), and retain the virus for a short period, normally less than 2 h (25). Efficient vectors of SPFMV are the aphid species *Myzus persicae* and *Aphis gossypii* (3,35). *M. persicae* occasionally colonizes sweetpotato in Louisiana; while *A. gossypii* has been found on *I. hederacea* (C. A. Clark, unpublished data).

Potyvirus spp. are detected in sweetpotato using various combined methods. Because they exist in very low titers in sweetpotato (19), graft indexing on *I. setosa* is traditionally used to detect its presence and subsequent testing using other techniques. The most widely used serological method (5) is a membrane immune-bind-

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ing assay known as nitrocellulose membrane enzyme-linked immunosorbent assay (NCM-ELISA). Reverse-transcription polymerase chain reaction (RT-PCR) and real-time RT-PCR are also used for detection and quantification (5,19).

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*.

Materials and Methods

Host plants. *I. nil* 'Scarlet O'Hara' (SOH) was used as the test plant in all studies. The following *Ipomoea* spp. were used as SPFMV acquisition sources: Beauregard and Evangeline sweetpotato, *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. *I. cordatotriloba* and *I. hederacea* were established from seed harvested from single plants that were grown in the greenhouse. All plants were grown in the greenhouse under wide temperature (10 to 32°C) and humidity (21 to 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 Osmocote 14-14-14 (Scotts-Sierra Horticultural Products Company) at 3.5 g/pot. Plants were not sprayed with insecticide. Establishment of sweetpotato plants from virus-tested mericlones and morning glory plants from seed ensured they were virus free before being used in studies.

Virus inoculum. Two inoculum sources were used in our transmission studies: plants infected with the RC strain of SPFMV (SPFMV-RC, isolate 95-2) maintained in SOH in the greenhouse by repeated mechanical inoculation, and a naturally mixed-infected Beauregard sweetpotato (B 14, G-7) that was grown in fields in North Carolina for 7 years and provided by G. C. Yencho (Department of Horticultural Sciences, North Carolina State University, Raleigh). B 14, G-7 was tested using NCM-ELISA after grafting on *I. setosa*, RT-PCR, and real-time 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* (SPCSV), *Sweet potato collusive virus*, and *Cucumber mosaic virus* (CMV). However, the possibility that it was infected by viruses not yet recognized in sweetpotato cannot be eliminated.

Aphid colonies. *A. 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 apterae and maintained under laboratory conditions in screened cages at room temperature (20 to 22°C) and a photoperiod of 16 h of light and 8 h of darkness. *A. gossypii* was reared on 'Stoneville 474' cotton plants (*Gossypium* spp.), while *M. persicae* was reared on 'Tendergreen' mustard plants (*Brassica cretica* L.), neither of which has been described as a host for sweetpotato viruses. Plants were grown in the greenhouse under wide temperature (10 to 32°C) and humidity (21 to 98%) ranges, 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.

NCM-ELISA assays. Leaf tissue was collected from symptomatic plants and assayed by NCM-ELISA (10) using antisera produced to the SPFMV-RC 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 (approximately 1 cm²) was collected from a lower, middle, and upper leaf of each plant, combined, placed in a "Universal" extraction bag (article number 430100; BIOREBA), and homogenized using a HOMEX6 homogenizer (BIOREBA) in 8 ml of Tris-buffered saline (TBS; 0.02 M Tris base and 0.50 M NaCl, pH 7.5, containing 0.2% sodium sulfite (Na₂SO₃). The extracted tissue (2 ml) was transferred to a 2-ml microfuge tube and allowed to stand for 30 to 45 min 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 min. 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. (10). The first antibody (polyclonal specific to SPFMV, SPVG, or SPV2) was cross absorbed in healthy *I. setosa* extract (1 g of tissue homogenized in 25 ml of TBS containing sodium sulfite at 2 g/liter, Carnation skim milk [Nestlé USA, Inc.] at 20 g/liter, and sodium azide at 0.2 g/liter; pH 7.5) for 45 min at 37°C and was then added to the membranes and incubated 1 h. Positive reactions were determined by visual assessment, and a purple color reaction was recorded as positive.

Establishment of virus acquisition and test plants. Virus-tested Beauregard and Evangeline sweetpotato 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. Because titers in sweetpotato are often too low for detection by ELISA (5,20), plants were assayed for successful inoculation by grafting onto the standard virus indicator plant, Brazilian morning glory (*I. setosa*), and only those that produced typical SPFMV symptoms were used for study. *I. 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) 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 Evangeline. In the case of 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.

Transmission experiments with *A. gossypii* and *M. 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 Beauregard and Evangeline sweetpotato for both single infection with SPFMV-RC isolate 95-2 and the naturally mixed infection.

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 h. Fresh leaves expressing the most distinct symptoms of SPFMV (except for single-infected Beauregard and Evangeline, on which all leaves were asymptomatic) were obtained from respective source plants 3 weeks after mechanical inoculation or 5 to 6 weeks after graft inoculation. Leaves corresponded to

those that have high or most consistent titer (E. N. Wosula, *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 s in preliminary tests and 30 s in subsequent tests, after which it was transferred to an individual test plant. In each experiment, the same 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 real-time RT-PCR. Individual viruliferous aphids were allowed an inoculation access period of 2 h, after which plants were sprayed with imidacloprid (Admire 2F; Bayer CropScience) at a rate of 0.9 g a.i./liter of 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 the presence of SPFMV. Another transmission experiment was conducted with both aphid species using a 3-s AAP on *I. hederacea* and *I. cordatotriloba* single-infected and Beauregard single and mixed-infected. 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 factors. Transmission efficiency of SPFMV was estimated based on number of plants exposed, and the number of those infected by maximum likelihood using a Microsoft Excel Add-In PooledInfRate, version 3.0 (2). Additional differences in pooled data for host plants, infection status, and aphid species were tested using GLM PROC GENMOD (SAS Software version 9.2).

Quantitative RT-PCR assays. The real-time RT-PCR assays were carried out according to procedures of Kokkinos and Clark (19). 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.) according to the manufacturer's directions. RNA concentrations were determined using a Nanodrop spectrophotometer (Nanodrop Technologies). To eliminate residual DNA, total RNA samples were treated with DNase I (Invitrogen). Real-time RT-PCR assays were performed in 25- μl reaction volume mixtures containing 900 nM each primer (forward and reverse); 200 nM MGB TaqMan probe; 12.5 μl of 2 $\times$ Master mix; 0.63 μl of RT enzyme mix (40 $\times$), which contains MultiScribe Reverse Transcrip-

tase and RNase Inhibitor respectively, of the TaqMan One-Step PCR Master Mix Reagents kit (Applied Biosystems); 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). The following real-time RT-PCR thermal cycling conditions were used: 48°C for 30 min (cDNA synthesis), 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing and extension at 60°C for 1 min. All real-time RT-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 fivefold 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, 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 $N = 2^{-\Delta\text{Ct}}$, where ΔCt is the difference between the Cts of the target virus and endogenous control (18S RNA) obtained from their respective real-time 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). Data were log transformed and analyzed using PROC GLM, PROC Means, and PROC Corr procedures (SAS Software version 9.2).

Results

Transmission experiments with *A. gossypii* and *M. persicae*.

The only successful transmission from sweetpotato was with *A. gossypii* from mixed-infected Beauregard for both 3- and 30-s acquisitions (Tables 1 and 2). SPFMV-RC transmission from mixed- compared with single-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 1). *M. persicae* yielded no significant differences in transmission from mixed- compared with single-infected sources within host plant species. No successful transmission of SPFMV occurred with Evangeline as source from either mixed- or single-infected or from single-infected Beauregard (Tables 1 and 2). The 3-s probe yielded successful transmissions of SPFMV in some host-virus combinations by both aphid species, though at lower rates compared with the 30-s probes (Tables 1 and 2). According to the NCM-ELISA results, all test plants whose virus sources were mixed-infected tested positive for SPFMV. All transmissions by *M.*

Table 1. Sweet potato feathery mottle virus (SPFMV) transmission from *Ipomoea* spp. from single and mixed infections by *Aphis gossypii* and *Myzus persicae* following 30-s acquisition probes^a

Acquisition source ^b	Transmission (%)			
	<i>A. gossypii</i>		<i>M. persicae</i>	
	Mean	CI	Mean	CI
<i>Ipomoea 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

^a Means within a column (acquisition sources) with same confidence interval (CI) range are not significantly different ($P > 0.05$). NT = no transmission occurred; therefore, no CIs can be calculated.

^b RC = russet crack strain; $n = 100$ for each acquisition source.

persicae tested positive for SPFMV alone, whereas some transmissions by *A. gossypii* from all hosts tested positive for both SPFMV and SPVG and none tested positive for SPVG alone (Table 3). *A. gossypii* had a significantly greater transmission efficiency compared with *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 with other hosts ($\chi^2 = 20.66$, $P \leq 0.0001$), and mixed-infected leaves were better SPFMV sources than single-infected ones ($\chi^2 = 8.78$, $P \leq 0.0030$).

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 single-infected plants, but *I. cordatotriloba* mixed-infected had a significantly lower titer than in single-infected. No significant differences between mixed- and single-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* single-infected morning glory plants than in the sweetpotato Beauregard and Evangeline (Fig. 1). Correlation analysis revealed a significantly positive relationship between transmission and virus titer for both *A. gossypii* and *M. persicae* ($r = 0.74$, $P = 0.0131$ and $r = 0.82$, $P = 0.0347$, respectively).

Discussion

Dramatic differences in transmission of SPFMV occurred from different source plants depending on both host species and infection status. Based on our 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 Beauregard or Evangeline sweetpotato. Significantly greater SPFMV titers occurred in morning glory plants compared with sweetpotato plants. We suggest that SPFMV replication in morning glory plants is more rapid compared with sweetpotato; hence, high titers occur that enable easier acquisition and transmission by aphids. Kennedy and Moyer (17) 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 vi-*

rus; 1,4,34). 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* (12). There was a positive relationship between virus transmission and titer levels by both *A. gossypii* and *M. persicae* (31).

SPFMV was transmitted at a greater rate across all host species for *A. gossypii* from mixed-infected than single-infected plants. Virus titers did not differ between mixed- and single-infected plants within species except that titers were higher for mixed-infected than single-infected Beauregard and single-infected than mixed-infected *I. cordatotriloba*. Our results suggest that 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 with single infections, indicating that there could be other contributing factors apart from virus titer. Kokkinos and Clark (20) reported significantly greater titers of SPFMV in sweetpotato plants when co-infected with a *Crinivirus* sp. SPCSV than in single-infected plants. However, there was no significant difference in SPFMV titer in single and co-infections with two other sweetpotato *Po-*

Fig. 1. Relative titers of Sweet potato feathery mottle virus russet crack strain (SPFMV-RC) and frequency of SPFMV transmission by *Aphis gossypii* and *Myzus persicae* in singly and mixed infected host plants (lh = *Ipomoea hederacea*, lc = *I. cordatotriloba*, B = Beauregard sweetpotato, E = Evangeline sweetpotato, s = singly infected, m = mixed infected).

Table 2. Sweet potato feathery mottle virus (SPFMV) transmission from *Ipomoea* spp. from single and mixed infections by *Aphis gossypii* and *Myzus persicae* following 3-s acquisition probes^a

Acquisition source ^b	Transmission (%)			
	<i>A. gossypii</i>		<i>M. persicae</i>	
	Mean	CI	Mean	CI
<i>Ipomoea 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

^a Means within a column (acquisition sources) with same confidence interval (CI) range are not significantly different ($P > 0.05$). NT = no transmission occurred; therefore, no CIs can be calculated.

^b RC = russet crack strain; $n = 100$ for each acquisition source.

Table 3. Frequency of detection of Sweetpotato feathery mottle virus (SPFMV) and Sweet potato virus G (SPVG) in test plants following *Aphis gossypii* and *Myzus persicae* transmission from mixed-infected hosts following a 30-s acquisition probe^a

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

^a For each acquisition source, $n = 100$; — indicates not tested due to lack of successful transmission.

tyvirus spp., SPVG and SPV2 (C. D. Kokkinos and C. A. Clark, *unpublished data*). Therefore, our significant differences between mixed- and single-infected virus titers in Beauregard suggest that 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 (36). 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 (15) reported increased transmission of *Alfalfa mosaic virus* (AMV) by the pea aphid *Acyrtosiphon pisum* when co-infected with *Pea streak virus* (PSV) compared with single-infected plants. However, they observed the reverse trend with PSV, whose transmission efficiency was lower when co-infecting with AMV than when alone. Our results are contrary to those of Pinto et al. (26), who observed reduced transmission of *Zucchini yellow mosaic virus* (ZYMV), *Cucumber mosaic virus* (CMV), and *Papaya ring spot virus* from mixed infections compared with single-infected plants by both *Aphis gossypii* and *M. persicae*. Wintermantel et al. (42) also reported reduced transmission of two *Crinivirus* spp. when co-infecting compared with single infections. Significant reduction of SPFMV titer in mixed- compared with single-infected *I. cordatotriloba* could be attributed to host effect on virus titer accumulation pattern. Some viruses may have enhanced or reduced titer in co-infections versus single infections depending on host plant species or cultivar (36,42).

Overall, *A. gossypii* had a significantly greater transmission efficiency than *M. persicae*. *A. 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 our results under laboratory conditions suggest that *A. gossypii* is a more efficient vector of SPFMV, field conditions present a different case, because the importance of a vector is determined based on its propensity, a measure determined by both vector efficiency and abundance (16). Preliminary data on aphids caught in Louisiana sweetpotato fields using yellow sticky and pan traps indicate that *A. gossypii* is more abundant than *M. persicae*. Single or multiple virus species or strains can be transmitted from mixed-infected hosts depending on the aphid species (30). Several publications have reported differences in virus transmission by various aphid species (17,41). These differences in transmission are attributed to interaction of virus particles, helper component protein, surface proteins on the aphid stylet, and virus coat protein that affect virus binding capacity and vector behavior (25,36). The transmission efficiency of SPFMV by *M. persicae* (0 to 18%) in our experiments was comparable with what has been reported for some *Potyvirus* spp.; for example, PVY, 4.7 to 71.1% (4,11,28); *Soybean mosaic virus*, 18.6%; AMV, 5 to 19%; and ZYMV, 20 to 60% (37). Transmission by *A. gossypii* was comparable with what has been reported on other *Potyvirus* spp. (for example, ZYMV, 27.5%; 43). Successful transmission of SPFMV by *M. persicae* and *A. gossypii* in as few as 3 s reveals how easily this virus can be acquired and spread in the fields by these aphid species. Earlier studies by McLean (23) revealed a similar trend.

Our findings demonstrate that transmission of SPFMV depends on host plant, aphid species, and infection status. The knowledge of 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 *Potyvirus* spp. 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 glory plants germinate from true seed that are not initially infected. Preliminary observations indicate that the morning glory plants get infected during the

months of June to July when SPFMV is already spreading among sweetpotato plants (E. N. Wosula, *unpublished*). 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 elevated secondary sources of SPFMV and, hence, 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. Preliminary results of a field study suggest that virus titers may vary dramatically under field conditions and may differ from greenhouse conditions (E. N. Wosula, *unpublished*). The fact that SPFMV was acquired quickly and transmitted in a nonpersistent manner also suggests that mineral or stylet oils, which have been reported to minimize virus spread of nonpersistent aphid-borne viruses (32), should be evaluated for their potential to inhibit transmission of SPFMV, as another step toward devising integrated strategies to minimize virus spread. Further work is required to determine whether lack of transmission from Evangeline sweetpotato 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 with Beauregard, and SPFMV titers are lower in Evangeline compared with Beauregard in field samples (E. N. Wosula, *unpublished*).

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