Bemisia afer sensu lato, a Vector of *Sweet potato chlorotic stunt virus*

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ABSTRACT

Gamarra, H. A., Fuentes, S., Morales, F. J., Glover, R., Malumphy, C., and Barker, I. 2010. *Bemisia afer* sensu lato, a vector of *Sweet potato chlorotic stunt virus*. Plant Dis. 94:510-514.

Bemisia tabaci biotype B is considered to be the primary vector of *Sweet potato chlorotic stunt virus* (SPCSV, *Crinivirus*). However, *Trialeurodes abutiloneus* also has been shown to transmit SPCSV in a semipersistent manner. Mixed infection of SPCSV with the aphid-transmitted *Sweet potato feathery mottle virus* (SPFMV, *Potyvirus*) causes sweetpotato (*Ipomoea batatas*) virus disease (SPVD), the major virus disease affecting this crop. High populations of *B. afer* sensu lato are seasonally associated with sweetpotato in Peru during times of low *B. tabaci* incidence. The transmission of SPCSV (in single and double infection with SPFMV) by laboratory-reared *B. afer* sensu lato and *B. tabaci* biotype B was investigated. For SPCSV transmission efficiency, individual adult insects were allowed 48 h for acquisition and inoculation access periods at both 20 and 25°C. SPCSV was transmitted by both whiteflies, with similar transmission efficiency when the virus was acquired from plants singly infected by SPCSV or doubly infected with SPCSV and SPFMV, at 20 and 25°C. We conclude that *B. afer* sensu lato is a newly identified vector of SPCSV. This finding may have important epidemiological significance for the spread of SPCSV and SPVD.

Sweet potato chlorotic stunt virus (SPCSV) is a *Crinivirus* sp. (family *Closteroviridae*) transmitted by *Bemisia tabaci* (Gennadius) and *Trialeurodes abutiloneus* Haldeman (Hemiptera: Aleyrodidae) (21) in a semipersistent manner (4,10,27,34, 40,44,45). SPCSV is the most important virus affecting sweetpotato (*Ipomoea batatas* (L.) Lam.) due to its ability to mediate severe synergistic diseases with several other sweetpotato-infecting viruses belonging to different genera (14,15,26,39). SPCSV, together with the aphid-borne *Potyvirus* sp. *Sweet potato feathery mottle virus* (SPFMV; family *Potyviridae*), are the causal agents of sweetpotato virus disease (SPVD), the main viral disease affecting this crop in different regions of the world (11,14,29,38). Sweetpotato yield reductions caused by SPCSV are approximately 30% but can exceed 50% when interacting with other viruses (11,17).

B. afer (Priesner & Hosny) (3,6) sensu lato occurs in Africa, Australia, the Mediterranean coast of Europe, southern England, and South America (3,18,20). This whitefly species was first reported in the Americas in Peru on sweetpotato in 2000 (3). *B. afer* infests plants in the families *Anacardiaceae*, *Annonaceae, Apocynaceae, Bignoniaceae, Bixaceae, Bombacaceae, Burseraceae, Celastraceae, Caprifoliaceae, Combretaceae, Convolvulaceae, Euphorbiaceae, Fabaceae, Labiateae, Liliaceae, Loganiaceae, Lythraceae, Malvaceae, Moraceae, Myrtaceae, Papaveraceae, Rhamnaceae, Rosaceae, Rubiaceae, Rutaceae*, *Salicaceae, Sapindaceae, Solanaceae*, and *Urticaceae* (3,5,16,20,24).

B. afer and SPCSV have been previously reported together in Peru, Uganda, Kenya, Tanzania, Madagascar, Nigeria, Egypt, and Spain (3,11,17). Although *Crinivirus* spp. are unique among whitefly-vectored viruses in that members of different genera of whiteflies can transmit them, *B. tabaci* biotype B is the only *Bemisia* sp. identified as a vector of SPCSV to date. The possibility that *B. afer* sensu lato is a vector for this virus is particularly relevant because this species of whitefly colonizes sweetpotato at high levels. The importance of *B. afer* sensu lato as a vector of sweetpotato viruses (or any other plant viruses) has never been documented.

In this study, we describe, for the first time, transmission of SPCSV by *B. afer* sensu lato, the effect of temperature on the transmission of SPCSV in single and double infections with SPFMV, and the epidemiological implications associated with efficient transmission of SPCSV by both *B. afer* sensu lato and *B. tabaci* biotype B.

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Accepted for publication 10 January 2010.

doi:10.1094/PDIS-94-5-0510

MATERIALS AND METHODS

Plant material and virus isolates. Virus-free sweetpotato cv. Costanero plants, determined by indexing them through grafting to *I. setosa* Ker with subsequent serological tests, were provided by the International Potato Center (CIP, Lima, Peru). Virus-free plants of *I. nil* (L.) Roth were grown from botanical seed, because no sweetpotato virus reported so far is seed transmitted. In addition, random samples were tested by enzyme-linked immunosorbent assay on nitrocellulose membranes (NCM-ELISA) (11) to confirm freedom from SPCSV and SPFMV. Hereafter, virus-free plants are referred to as "healthy plants."

SPCSV isolate M2-47, belonging to the East African (EA) strain (11), was obtained from infected sweetpotato plants collected in the Valley of Cañete, Peru, using *B. tabaci* biotype B as vector on *I. nil*.

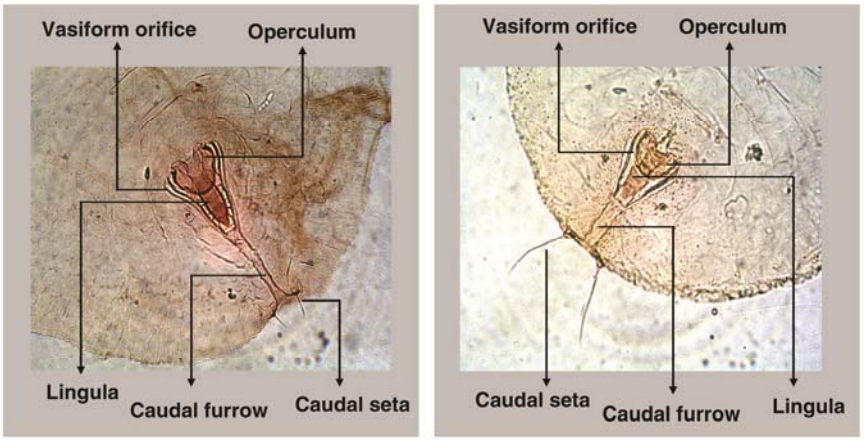
The russet crack strain of SPFMV (U.S. isolate) was obtained from the CIP virus collection and maintained in *I. nil* by mechanical inoculation.

Healthy sweetpotato plants were side-graft inoculated with SPCSV (single infection) and with both SPCSV and SPFMV (double infection) to yield virus sources for transmission studies.

Whitefly colonies and species identification. CIP colonies of *B. afer* sensu lato and *B. tabaci* biotype B were established from the pupal stage obtained from sweetpotato plants growing in the Valley of Cañete, Lima, Peru. The whiteflies were mass-reared on healthy sweetpotato cv. Costanero plants. The third generation was used to obtain whitefly colonies of the same age. Identification of the whitefly species was confirmed morphologically from the puparia (Fig. 1) (20,30) in two different laboratories: the International Center for Tropical Agriculture (CIAT), Colombia and The Food and Environment Research Agency (FERA), UK. The two whitefly species were also differentiated by amplification and sequencing of a fragment of the mitochondrial 16S rDNA gene, obtained by polymerase chain reaction (PCR) at CIAT-Colombia. Universal primers 4118 (CCGGTCTGAACTCAG ATCACGY) and 4119 (CGCCTGTTT AACAAAAAACAT), constructed for the mitochondrial 16S rDNA gene of *Drosophila yakuba*, were used in the PCR reactions according to Xiong and Kocher (46) and modified by Calvert et al. (8).

For *B. afer* sensu lato, the mitochondrial 16S rDNA fragment obtained by PCR was sequenced and compared with that published in the GenBank database for *B. leakii* Peal (AF247531); *B. hancocki* Corbett (AF247532); *B. tabaci* biotypes B, Q,

and S (AF246636, AF246647, and AF247527, respectively); *B. tabaci* biotypes collection (AF110722, AF110714, AF110713, AF110715, AF110716, AF110717, AF110719, and AF110721), *T. vaporariorum* Westwood (AF110723); *Aleurotrachelus europlatus coronata* Quaintance (EU471164); and those unpublished provided by CIAT for *B. tabaci* biotype A, *B. tuberculata* Bondar, *T. vaporariorum*, *T. variabilis* Quaintance, and *Aleurotrachelus socialis* Bondar (8). The alignments and phylogenetic analysis were performed with the MEGA 4 software package (37). Distances were calculated using the Kimura two-parameter model, and a tree was assembled using neighbor joining with 2,000 bootstrap replicates.



Morphological character	*Bemisia afer*	*Bemisia tabaci* biotype B
Caudal setae	Always less than half the length of the vasiform orifice and often minute; highly variable among individuals	Always stout and usually as long or longer than vasiform orifice. Little variation among individuals
Vasiform orifice	Usually shorter than length of the caudal furrow, with sides often distinctly concave. The surface of vasiforme orifice underneath lingula is reticuled. The vasiform orifice is concave	Slightly longer than the length of the caudal furrow, with straight sides. The surface of vasiforme orifice underneath lingula in *B. tabaci* is not reticulated compared with *B. afer*. The orifice is triangular in shape.
Lingula	Long and narrow	Shorter and slightly wider than in *B. afer*
Caudal furrow	Similar in length to the vasiform orifice	Slightly shorter than the length of the vasiform orifice
Operculum	The operculum is triangular in shape	The operculum is oval in shape

Fig. 1. Comparison of some morphological characters of *Bemisia tabaci* biotype B and *B. afer* sensu lato puparia, used for taxonomic identification. Top, photographs of the posterior of the puparia. Bottom, descriptions of morphological characters of the puparia.

Virus transmission. Completely randomized experimental design was set up as a 2^3 factorial (two whitefly species, two temperatures, and two virus sources) in four replications with 25 plants per experimental unit (Table 1). Adult whiteflies from the third generation of *B. afer* sensu lato and *B. tabaci* biotype B were used as vectors in the virus transmission efficiency tests. Individual whitefly adults were allowed an acquisition access period (AAP) of 48 h on sweetpotato plants infected with SPCSV and with both SPCSV and SPFMV. Single viruliferous whiteflies were then placed on individual healthy *I. nil* plants (test plants) for an inoculation access period (IAP) of 48 h (Table 1). Acquisition and inoculation periods were carried out at both 20 and 25°C in a growth chamber (temperature controlled, with 3,500 lx per 12 h of fluorescent and bulb lights).

Five infected *I. batatas* plants with high levels of SPCSV (from single and double infection with SPFMV), as determined by NCM-ELISA (11), were selected as virus sources. At the end of each IAP, whiteflies were removed manually and plants were sprayed with 1% buprofezin. Inoculated *I. nil* plants were maintained in an insect-proof greenhouse at 10,000 to 15,000 lx light intensity for evaluation. Number of infected plants was recorded and transmission efficiency was analyzed by the test of equal or given proportions using the R Statistical program (31). For interaction studies, normality of data was corrected through angular transformation, which allowed performance of analysis of variance.

Virus detection. Test plants were evaluated beginning 2 weeks after inoculation and monitored for 60 days. SPCSV infection was initially detected by symptom expression in the inoculated plants at 10 to 20 days after inoculation. All symptomatic

Table 1. Transmission efficiency of *Sweet potato chlorotic stunt virus* (SPCSV) with individual adults of *Bemisia afer* sensu lato and *B. tabaci* biotype B at two temperatures, using sweetpotato (*Ipomoea batatas*) cv. Costanero plants infected with SPCSV (single infection) and with SPCSV and *Sweet potato feathery mottle virus* (SPFMV) (double infection) as virus source plant and *Ipomoea nil* as test plant

	Temperature													
	20°C							25°C						
	Replications[a]							Replications[a]						
Whitefly, virus source	1	2	3	4	E (%)[b]	STD[c]	SE[d]	1	2	3	4	E (%)[b]	STD[c]	SE[d]
B. afer sensu lato														
SPCSV	3/23	2/25	2/25	4/25	11.2	0.96	0.24	4/25	2/25	2/25	5/25	13	1.5	0.38
SPCSV+SPFMV	1/25	3/24	2/25	4/25	10.1	1.29	0.32	1/25	2/25	3/24	1/25	6.1	0.96	0.24
B. tabaci biotype B														
SPCSV	4/25	2/25	0/25	1/25	7	1.71	0.46	3/25	2/25	0/25	3/25	8	1.41	0.35
SPCSV+SPFMV	2/25	4/25	4/24	3/25	13.1	0.96	0.24	2/24	6/25	0/25	4/25	12.1	2.58	0.65

[a] Number of infected plants/number of total inoculated plant (experimental unit) per each replication.
[b] Percentage of total infected plants from total inoculated plants.
[c] STD = standard deviation.
[d] SE = standard error.

and nonsymptomatic plants were tested by NCM-ELISA (11) or by reverse-transcription (RT)-PCR) periodically throughout the 60 days. Primers CP1 (CGTCTAGATTGTTAGAAA) and CP3 (AACGCG GAAGTGTAAGGTAT) were used in the RT-PCR reactions according to Alicai et al. (2). Total nucleic acids were extracted from plants prior to RT-PCR using Plant RNA Purification Reagent (Invitrogen, CA).

RESULTS

Identification of whiteflies species. Both species of *Bemisia* were identified by the morphological characters observed on puparia, according to the key features shown in Figure 1. Pupae of *B. afer* sensu lato are larger and are transparent or slightly yellowish compared with those of *B. tabaci*. Important diagnostic characteristics of *B. afer* sensu lato include short caudal setae, vasiform orifice subequal in length to caudal furrow, and lingual head long and narrow with triangular operculum (Fig. 1). The CIP colony was confirmed as *B. afer* sensu lato by whitefly taxonomists P. Hernandez at CIAT and C. Malumphy at FERA. A 529-bp PCR product amplified from the mitochondrial 16S rDNA was obtained from *B. afer* samples. Phylogenetic analysis of the *B. afer* sensu lato nucleotide sequence (GenBank accession no. FJ969473) indicated a relatively close relationship to *B. tuberculata, B. leakii,* and *B. hancocki* (84.1, 82.8, and 81.9% identity, respectively) (Fig. 2).

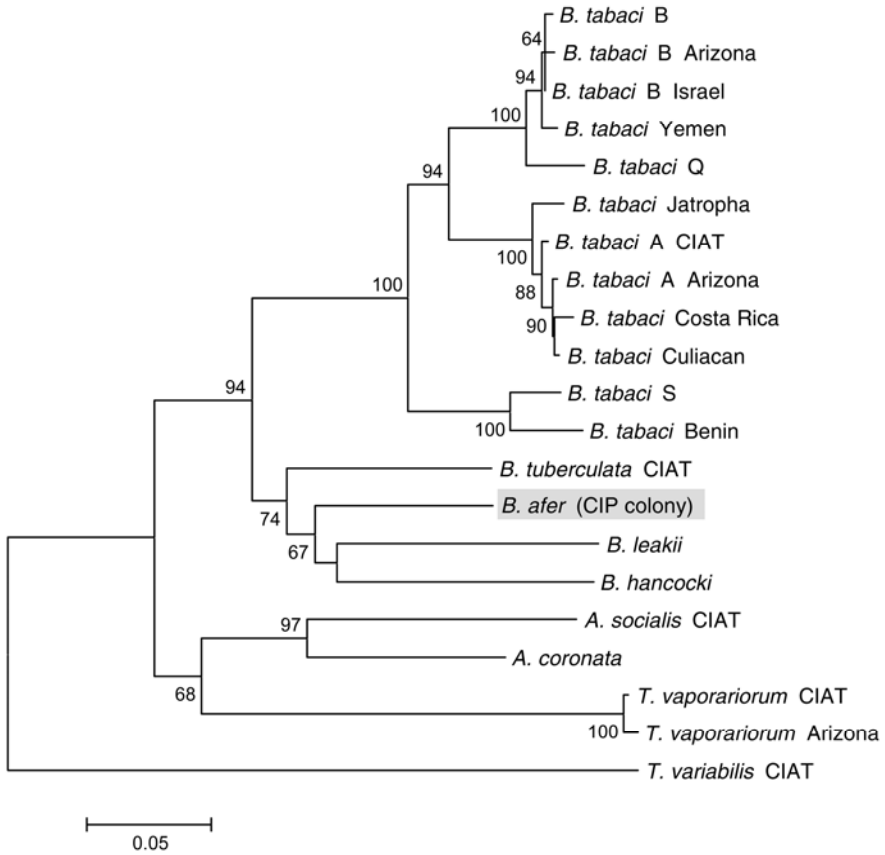


Fig. 2. Phylogenetic tree calculated from alignments of nucleotide sequences on the mitochondrial 16S rDNA gene of *Bemisia afer* sensu lato (GenBank accession no. FJ969473) with other whitefly species. Percentage of bootstrap support out of 2,000 replicates is given at each of the major nodes in the tree. Scale bar indicates Kimura nucleotide distances.

Virus transmission. All plants exhibiting symptoms of SPCSV tested positive for SPCSV by NCM-ELISA and RT-PCR, and all symptomless plants tested negative. SPCSV was transmitted at a low level by individual whiteflies of both *B. afer* sensu lato (6.1 to 13%) and *B. tabaci* biotype B (7 to 13.1%) when the virus was acquired from singly or doubly infected *I. batatas* plants (Table 1). The transmission rate of the virus was higher when transmitted by *B. afer* sensu lato from singly than doubly infected plants, with this difference being more evident at 25 than at 20°C (Table 1). The contrary occurred with *B. tabaci* biotype B, with an apparent increased rate of transmission from doubly infected plants at both temperatures (Table 1). However, none of these differences are statistically significant (Table 2). Both whitefly species transmitted the virus with similar transmission efficiency because they did not differ significantly in their transmission rate when SPCSV was acquired from singly infected or doubly infected plants or when comparing both virus-infected sources plants at both temperatures (Table 2).

The interaction between whitefly species and virus sources was moderately significant (P value = 0.09) in spite of a relatively high variation coefficient of 43%.

DISCUSSION

Knowing the existence of new virus vectors is important for plant disease management. This study provides experimental data showing that, in addition to *B. tabaci* and *T. abutiloneus*, SPCSV can also be transmitted by the whitefly species *B. afer* sensu lato. To our knowledge, this is the first time that *B. afer* sensu lato has been reported as a virus vector. Previous studies by Maruthi et al. (22) reported that *B. afer* sensu lato was not able to transmit the *Ipomovirus* sp. *Cassava brown streak virus*, even though the disease seemed asso-

Table 2. Statistical analysis by the test of equal or given proportion (n = 98 to 100) of the transmission of *Sweet potato chlorotic stunt virus* (SPCSV) from two virus sources by two whitefly species at two temperatures, using the R Statistical program (31)

	at 20°C		at 25°C		*B. afer* sensu lato		*B. tabaci* biotype B	
Comparison of	**χ^2**	***P* value**	**χ^2**	***P* value**	**χ^2**	***P* value**	**χ^2**	***P* value**
Virus source for[a]								
B. afer sensu lato	<0.001	0.98	1.33	0.25	…	…	…	…
B. tabaci biotype B	1.47	0.23	0.53	0.46	…	…	…	…
Vector species from[b]								
SPCSV+SPFMV source	0.19	0.66	0.93	0.33	…	…	…	…
SPCSV source	0.62	0.46	0.85	0.36	…	…	…	…
Temperatures with[c]								
SPCSV+SPFMV source	…	…	…	…	0.26	0.61	0.00	1.00
SPCSV source	…	…	…	…	0.03	0.87	0.00	1.00

[a] Comparison of virus source: SPCSV versus *Sweet potato feathery mottle virus* (SPFMV).
[b] Comparison of vector species: *B. afer* sensu lato versus *B. tabaci* biotype B.
[c] Comparison of temperatures: 20 versus 25°C.

ciated with this species in the field. In the study presented herein, transmission of SPCSV was achieved by both *B. afer* sensu lato and *B. tabaci* biotype B. The transmission of SPCSV by individual adults of *B. afer* sensu lato suggests that this insect is a relatively efficient vector of SPCSV. Wintermantel and Wisler (42) observed that only the most efficient vectors were capable of transmitting the closely related *Crinivirus* sp. *Tomato chlorosis virus* (ToCV) by individual whiteflies. It has been also observed that transmission efficiency of the *Crinivirus* spp. ToCV and *Lettuce infectious yellows virus* are influenced by the number of individuals used to inoculate test plants (27,28,42). Obviously, the number of whitefly individuals found on plants under field conditions greatly exceeds the highest numbers of whitefly individuals tested under experimental conditions.

It seems possible that whiteflies might acquire the virus more readily from hosts other than sweetpotato (33). AAP and IAP of 48 h, fairly typical for *Crinivirus* spp., have been shown to work well for transmission of SPCSV (34,40). In this study, the transmission of SPCSV by single whiteflies of both *B. afer* sensu lato and *B. tabaci* biotype B was low from infected sweetpotato plants. It was reported that transmission efficiency of sweetpotato viruses increases when a larger number of whiteflies (more than 15) (12,32,33) are used per plant and when infected plants other than sweetpotato are used as the virus source.

Distinct isolates of SPCSV have been reported from different geographical regions (1,9,13,36). These isolates form two groups according to their serological and molecular relationship: the EA and the non-EA strains. *B. tabaci* biotype B is able to transmit SPCSV isolates from EA (this study) and non-EA (34) strains with similar efficiency. In both cases, transmission experiments were carried out using similar conditions: virus was acquired from SPVD-infected (SPCSV+SPFMV) sweetpotato by single whiteflies, with AAP and IAP of 48 h. In our studies, we found no significant differences in transmission efficiencies of SPCSV by *B. afer* sensu lato and *B. tabaci* biotype B at 20 or 25°C when acquired from singly or doubly infected plants. These results were similar to those obtained by Valverde et al. (40), who reported similar transmission rates for a non-EA isolate of SPCSV with *B. tabaci* biotype B from either single or double infections of SPCSV and SPFMV. It is known from previous reports (9,14,15,26) that SPCSV titers may significantly decrease in double infections with SPFMV. We did not estimate the SPCSV titer in the source plants in single and double infections with SPFMV. However, we observed that the number of SPCSV-infected plants was lower when the virus was transmitted by *B. afer* sensu lato from doubly infected plants than from singly infected plants. This is interesting, and it seems to be in line with the previous reports, suggesting that SPCSV titer was somewhat lower in double infections. Lower transmissions of the *Crinivirus* spp. ToCV and LIYV by whiteflies were also observed from source plants with lower virus concentration (27,28,42). In our study, the apparent increased rate in the transmission of SPCSV by *B. tabaci* biotype B from doubly infected plants seems contradictory to that expected with SPVD.

Most whitefly-transmitted viruses are transmitted by a single genus of whitefly. ToCV, a *Crinivirus* sp. closely related to SPCSV (43), is the best-known exception to this. ToCV has the ability to be transmitted by four whitefly vectors in two genera: *B. tabaci* biotypes A and B, *T. abutiloneus*, and *T. vaporariorum* (41,42,45), although efficiency and persistence differ among the vectors (42). In the present study, SPCSV was shown to be transmissible by vectors within both the *Bemisia* and *Trialeurodes* genera (34,40). Taking our findings into account, SPCSV, like ToCV, shares the distinction of transmissibility by three different whitefly species (*B. tabaci* biotype B, *B. afer* sensu lato, and *T. abutiloneus*) and, to the best of our knowledge, is the only *Crinivirus* sp. transmitted by a *Bemisia* sp. other than *B. tabaci*. Although we obtained a similar rate of transmission of SPCSV with both species of *Bemisia* (6.1 to 13.1%, respectively), it was higher than that reported for *T. abutiloneus* (3.2%) (34). Like other *Crinivirus* spp., SPCSV is not transmitted mechanically (7); therefore, it is dependent on whiteflies for plant-to-plant dissemination in the field.

The presence of *B. tabaci* biotype B, *B. afer* sensu lato, and *T. vaporariorum* on sweetpotato has been observed on the Peruvian coast. Because attempts to transmit SPCSV with *T. vaporariorum* have been unsuccessful (*unpublished data*), *B. afer* sensu lato and *B. tabaci* biotype B are likely to be the predominant vectors of SPCSV in Peru. Temperature is one of the main environmental factors affecting whitefly population dynamics (23). In the Cañete Valley, *B. afer* sensu lato predominates in sweetpotato fields after September, accounting for 99% of the whiteflies in November and December (25). Before September, *B. tabaci* biotype B is the dominant whitefly species in sweetpotato. The shift in whitefly population structure parallels changes in seasonal temperatures. It suggests that, in the winter and spring seasons (cooler temperatures), *B. afer* becomes the primary vector for SPCSV in the Cañete Valley, whereas *B. tabaci* predominates in the summer and fall (25) seasons, when temperatures are warmer, thus facilitating the dissemination of SPCSV and, subsequently, SPVD all year round.

B. hancocki has been synonymized with *B. afer* (6) but there remains doubt concerning this synonymy (19). The phylogenetic analysis of the nucleotide sequence indicated that *B. afer* sensu lato is different but closely related to *B. hancocki, B. leakii*, and *B. tuberculata* (81.9 to 84.1% nucleotide identity). This finding is not surprising because the *B. leakii* group is a taxonomically unresolved complex that contains at least three described species: *B. leakii*, *B. afer*, and *B. hancocki* (35). The *B. leakii* group is probably more confused than the *B. tabaci* group (35). The *B. leakii* group has been reported in India, Fiji, Tahiti, Papua New Guinea, American Samoa, Marshall Islands, Nauru, Palau, Tonga, and Vanuatu; *B. hancocki* in Africa and southern Europe; and *B. tuberculata* in Ecuador, Peru, Colombia, Venezuela, Brazil, Nicaragua, Puerto Rico, Costa Rica, and the Dominican Republic. *B. tuberculata* seems to be the vector of the *Begomovirus* spp. causing cassava mosaic disease and the agent of the cassava frog skin disease (5). This suggests that species other than *B. tabaci* can transmit *Begomovirus* spp.

B. afer sensu lato is a newly identified vector of SPCSV, the most important virus component of SPVD, and transmission rates seems to be sufficient to allow for disease spread. Because *B. afer* sensu lato outnumbers *B. tabaci* biotype B during the cooler season in Peruvian sweetpotato fields, it is likely to be the primary vector of SPCSV during those periods. *B. tabaci* biotype B is likely to be the predominant vector during the warmer seasons, when it becomes the predominant species. This observation has important epidemiological consequences for the management of sweetpotato virus diseases in Peru and other areas where both *B. afer* sensu lato and SPCSV are present in sweetpotato crops. The *B. afer* group exhibits considerable puparial morphological plasticity; therefore, we are referring to this species as *B. afer* sensu lato, because it may not be conspecific with the *B. afer* found in Europe, Africa, and Australia.

ACKNOWLEDGMENTS

This research was partly funded by the Tropical Whitefly Integrated Pest Management Project, funded by the Department for International Development of the UK (DFID). We thank F. de Mendiburu (Research Informatics Unit at CIP) for support with the statistical analysis of data; L. F. Salazar, C. A. Clark, and R. A. Valverde for critically reviewing this manuscript; and the International Center for Tropical Agriculture for helping in the morphological identification and molecular differentiation of whiteflies species and for providing information on sequences for different whiteflies species as indicated in the Materials and Methods.

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