Bemisia afer sensu lato, a vector of Sweet potato chlorotic stunt virus[1]

Heidy A. Gamarra[1], **Segundo Fuentes**[1], Francisco J. Morales[2], Rachel Glover[3], Chris Malumphy[3] and Ian Barker[1]

[1]International Potato Center, Av. La Molina 1895. La Molina, Lima, Peru; [2] International Center for Tropical Agriculture, Apartado Aéreo 6713, Cali, Colombia; [3]The Food and Environment Research Agency, Sand Hutton, York, U.K.
Corresponding author: Segundo Fuentes - s.fuentes@cgiar.org

Abstract

Bemisia tabaci biotype B is considered the primary vector of *Sweet potato chlorotic stunt virus* (SPCSV, *Crinivirus*). However, *Trialeurodes abutiloneus* also has been shown to transmit SPCSV. Mixed infection of SPCSV with the aphid-transmitted *Sweet potato feathery mottle virus* (SPFMV, *Potyvirus*) causes sweetpotato virus disease (SPVD), the major virus disease affecting this crop. High populations levels of *Bemisia 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 48h for acquisition and inoculation access periods at both 20°C 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°C and 25°C. We conclude that *B. afer* sensu lato is a new vector of SPCSV. This finding may have important epidemiological significance for the spread of SPCSV and SPVD.

Keywords: *Ipomoea batatas*, semi-persistent, whitefly transmission.

Sweet potato chlorotic stunt virus (SPCSV) is a crinivirus (Family *Closteroviridae*) transmitted by *Bemisia tabaci* (Gennadius) and *Trialeurodes abutiloneus* Haldeman (Hemiptera: Aleyrodidae) (21) in a semi-persistent manner (4,10,28,34,40,44,45). 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, *Sweet potato feathery mottle virus* (SPFMV; genus *Potyvirus*, family *Potyviridae*), are the causal agents of sweetpotato virus disease (SPVD), the main viral disease affecting this crop in different regions of the world (11,14,27,38). Sweetpotato yield reductions caused by SPCSV are ca. 30%, but can exceed 50% when interacting with other viruses (11, 17).

Bemisia 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). *Bemisia 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).

Bemisia afer and SPCSV have been previously reported in Peru, Uganda, Kenya, Tanzania, Madagascar, Nigeria, Egypt, and Spain (3,11,17). Although criniviruses 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* species 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.

[1] Accepted for publication in Plant Disease: July 03, 2009.

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.

Materials and methods

Plant material and virus isolates. Virus-free sweetpotato plants cv. Costanero, determined by indexing them through grafting to *I. setosa* Ker with subsequent serological test, were provided by the International Potato Center (CIP, Lima, Peru). Virus-free plants of *I. nil* (L.) Roth were grown from botanical seed, as no sweetpotato virus reported so far is seed-transmitted. In addition, random samples were tested by ELISA on nitrocellulose membranes (NCM-ELISA) (11) to confirm freedom of 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 (USA isolate) was obtained from 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 plants cv. Costanero. The third generation was used to get whiteflies colonies of the same age. Identification of the whitefly species was confirmed morphologically from the puparia as indicated in Fig. 1 (20,30) in two different laboratories: the International Center for Tropical Agriculture (CIAT), Colombia and The Food and Environment Research Agency (Fera), U.K. 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 (CCGGTCTGAACTCAGATCACGY) and 4119 (CGCCTGTTTAACAAAAACAT), 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, AF110721), *T. vaporariorum* Westwood (AF110723), *Aleuroplatus 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 2-parameter model, and a tree was assembled using neighbour joining with 2,000 bootstrap replicates.

Virus transmission. Completely randomized experimental design was set up as a 2^3 factorial (2 whitefly species, 2 temperatures and 2 virus sources) in 4 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°C and 25°C in a growth chamber (temperature controlled, with 3,500 lx / 12h of fluorescent and bulb lights).

Five infected 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 of them which allowed doing analysis of variance.

Virus detection. Test plants were evaluated beginning two 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 and non-symptomatic plants were tested by NCM-ELISA (11) and/or by reverse transcription-PCR (RT-PCR) periodically throughout the 60 days. Primers CP1 (CGTCTAGATTGTTAGAAA) and CP3 (AACGCGGAAGTGTAAGGTAT) 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, USA).

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 Fig. 1. Pupae of *B. afer* sensu lato are larger and are transparent or slightly yellowish compared to those of *B. tabaci*. Important diagnostic characters of *B. afer* sensu lato include short caudal setae, vasiform orifice subequal in length to caudal furrow, lingual head long and narrow, operculum triangular (Fig. 1). 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 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).

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 by both *B. afer* sensu lato and *B. tabaci* biotype B, with similar transmission efficiency (11.2 to 13% vs. 7 to 8%, respectively) when the virus was acquired from plants singly infected by SPCSV (Table 1 and 2). Similarly, when the SPCSV was acquired from plants doubly infected with SPCSV and SPFMV, *B. afer* sensu lato and *B. tabaci* biotype B did not differ significantly in their transmission of SPCSV (Table 2). No statistically significant differences in transmission of SPCSV were also observed in singly or doubly infected plants at 20°C and 25°C by both whitefly species (Table 2). However, the number of SPCSV-infected plants was higher when the virus was transmitted by *B. afer* sensu lato from singly infected plants than from doubly infected plants. The contrary occurred in the transmission of the virus by *B. tabaci* biotype B.

Interaction between whiteflies 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 *Cassava brown streak virus*, even though the disease seemed associated 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. Similar transmission rates were also obtained by Wintermantel and Wisler (42) with the closely related crinivirus *Tomato chlorosis virus* (ToCV) and individual whiteflies of *B. tabaci* biotype B and *T. abutiloneus*. They observed that the transmission efficiency of ToCV increased with increasing numbers of whiteflies per plant, and that only the most efficient vectors were capable of a high transmission rate using single whiteflies. Ng and Falk (28) and Ng et al. (29) demonstrated that whitefly transmission of the crinivirus *Lettuce infectious yellows virus* is influenced by the virus concentration in the source plant and the number of individuals used to inoculate test plants. Taken together, this suggests that *B. afer* sensu lato is a relatively efficient vector of SPCSV. 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). Acquisition and inoculation periods of 48 hours, fairly typical for criniviruses, 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 East African (EA) and the non-EA strains. *B. tabaci* biotype B is able to transmit SPCSV isolates from EA (this study) and non-EA (34) with similar efficiency. In both cases, transmissions experiments were carried out using similar conditions: virus was acquired from SPVD-infected (SPCSV+SPFMV) sweetpotato by single whiteflies, and 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°C or 25°C, when acquired from singly infected 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 infection and double infection with SPFMV. However, we observed that the number of SPCSV-infected plants was lower when the virus was transmitted by *B. afer* (but not by *B. tabaci* biotype B) from doubly infected plants than from singly infected plants. This is interesting and it seems to be in line with the previous reports, suggesting the SPCSV titer is somewhat lower in double infections.

Most whitefly-transmitted viruses are transmitted by a single genus of whitefly. *Tomato chlorosis virus* (ToCV), a crinivirus 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 only crinivirus transmitted by a *Bemisia* species other than *B. tabaci*. Although we obtained a similar rate of transmission of SPCSV with both species of *Bemisia* (6.1% to 13.1%), it was higher than that reported for *T. abutiloneus* (3.2%) (34). Like other criniviruses, 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 in the Peruvian coast. As 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 whitefly species predominates in sweetpotato. This shift in whitefly populations' 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.

Bemisia 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 since 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 group *B. leakii* is probably more confused than the *B. tabaci* group (35). *Bemisia leakii* group has been reported in India, Fiji, Thaiti, 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 Dominican Republic. *Bemisia tuberculata* seems to be the vector of the begomoviruses causing cassava mosaic disease and the agent of the cassava frog skin disease (5). This suggests that species other than *B. tabaci* can transmit begomoviruses.

Bemisia afer sensu lato is a new vector of SPCSV, the most important virus component of SPVD, and transmission rates seems to be sufficient to allow for disease spread. Since *B. afer* sensu lato outnumbers *B. tabaci* biotype B

during cooler season in Peruvian sweetpotato fields, it is likely the primary vector of SPCSV during those periods. *Bemisia tabaci* biotype B is likely 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 *B. afer* sensu lato is present in sweetpotato crops. Because *B. afer* group exhibits considerable puparial morphological plasticity, we are referring to this species as *B. afer* sensu lato, as it may not be conspecific with the *B. afer* found in Europe, Africa and Australia.

Acknowledgments

Authors thank Felipe 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. This research was partly funded by the Tropical Whitefly Integrated Pest Management Project, funded by the Department for International Development of the UK (DFID). The authors acknowledge 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.

Literature cited

Abad, J.A., Parks, E.J., New, S.L., Fuentes, S., Jester, W., and Moyer, J.W. 2007. First report of *Sweet potato chlorotic stunt virus*, a component of Sweetpotato virus disease, in North Carolina. Plant Dis. 91:327.

Alicai, T., Fenby, N.S., Gibson, R.W., Adipala, E., Vetten, H.J., Foster, G.D., and Seal, S.E. 1999. Occurrence of two serotypes of sweet potato chlorotic stunt virus in East Africa and their associated differences in coat protein and HSP70 homologue gene sequences. Plant Pathol. 48:718–726.

Anderson, P., Martin, J., Hernandez, P., and Lagnaoui, A. 2001. *Bemisia afer sens. lat.* (Homoptera: Aleyrodiadae) outbreak in The Americas. Florida Entomologist 84:316–317.

Aritua, V., Alicai, T., Adipala, E., Carey, E.E., and Gibson, R.W. 1998. Aspects of resistance to sweet potato virus disease in sweet potato. Ann. Appl. Biol. 132:387–398.

Bellotti, A.C., Smith L., and Lapointe, S.1999. Recent advances in cassava pest management. Ann. Rev. Entomol. 47:346–370.

Bink-Moenen, R.M. 1983. Revision of the African whiteflies (Aleyrodidae). Monografieen van de Nederlandse Entomologisch Vereninging, Amsterdam, No. 10. 211pp.

Brunt, A.A., Crabtree, K., Dallwitz, M.J., Gibbs, A.J., and Watson, L. (eds.). 1996. Viruses of Plants: Descriptions and Lists from the VIDE Database. CAB INTERNATIONAL, Cambridge, UK. 1484 pp.

Calvert, L.A., Cuervo, M., Arroyave, J.A., Constantino, L.M., Belloti, A., and Frohlich, D. 2001. Morphological and mitochondrial DNA marker analysis of whiteflies (Homoptera: Aleyrodidae) colonizing cassava and beans in Colombia. Ann. Entomol. Soc. Am. 94:512–519.

Cuellar, W.J., Tairo, F., Kreuze, J.F., and Valkonen, J.P.T. 2008. Analysis of gene content in sweet potato chlorotic stunt virus RNA1 reveals the presence of P22 protein RNA silencing suppressor in only few isolates: implications to viral evolution and synergism. J. Gen. Virol. 89:573–582.

Gibson, R.W., Mpembe, I., Alicai, T., Carey, E.E., Mwanga, R.O.M., Seal, S.E., and Vetten, H.J. 1998. Symptoms, aetiology and serological analysis of sweet potato virus diseases in Uganda. Plant Pathol. 47:95–102.

Gutiérrez, D., Fuentes, S., and Salazar, L.F. 2003. Sweetpotato virus disease (SPVD): distribution, incidence, and effect on sweetpotato yield in Peru. Plant Dis. 87:297–302.

Hollings, M., Stone, O.M., and Bock, K.R. 1976. Purification and properties of sweet potato mild mottle, a whitefly-borne virus from sweet potato *(Ipomoea batatas)* in East Africa. Ann. Appl. Biol. 82:511–528.

Hoyer, U., Maiss, E., Jelkmann, W., Lesemann, D.E., and Vetten, H.J. 1996. Identification of the coat protein gene of a sweet potato sunken vein closterovirus isolate from Kenya and evidence for a serological relationship among geographically diverse closterovirus isolates from sweet potato. Phytopathology 86:747–750.

Karyeija, R.F., Kreuze, J.F., Gibson, R.W., and Valkonen, J.P.T. 2000. Synergistic interactions of a potyvirus and a phloem-limited crinivirus in sweet potato plants. Virology 269:26–36.

Kokkinos, C. D., and Clark, C. A. 2006. Interactions among Sweet potato chlorotic stunt virus and different potyviruses and potyvirus strains infecting sweetpotato in the United States. Plant Dis. 90:1347–1352.

Lagnaoui, A. 2001. The whitefly complex (Homoptera:Aleyrodidae) affecting sweet potato in the Cañete Valle of Peru. International Symposium: Sweet potato Food and health for the future. Sec BII-O-11.

Loebenstein, G., Fuentes, S., Cohen, J. and Salazar, L. F. 2003. Sweet Potato. pp. 223–248 In: Virus and Virus-like Diseases of Major Crops in Developing Countries, Loebenstein, G and Thottappilly, G. (Eds). Kluber Academic Publishers, Dordrecht, The Netherlands.

Malumphy, C.P. 2003. The status of *Bemisia afer* (Priesner & Hosny) in Britain (Homoptera: Aleyrodidae). Entomologist's Gazette 54:191–196.

Martin, J.H. 1987. An identification guide to common whitefly pest species of the world (Homoptera, Aleyrodidae). Tropical Pest Management 33:298–322.

Martin, J.H., Mifsud, D., and Rapisarda, C. 2000. The whiteflies (Hemiptera: Aleyrodidae) of Europe and the Mediterranean basin. Bulletin of Entomology Research 90:407–474.

Martin, J.H. and Mound, L. 2007. An annotated check list of the world's whiteflies (Insecta: Hemiptera: Aleyrodidae). Zootaxa 1492:1–84.

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

Morales, F. and Jones, P. 2004. The ecology and epidemiology of whitefly-transmitted viruses in Latin America. Virus Res. 100:57–65.

Mound, L.A. and Halsey, S.H. 1978. Whitefly of the World: A systematic catalogue of the Aleyrodidae (Homoptera) with host plant and natural enemy data. British Museum (Natural History): Chichester. 329pp.

Mujica, N., Zegarra, O., and Lagnaoui, A. 2002. Ocurrencia estacional de las moscas blancas *Bemisia tabaci* y *Bemisia afer* en el Valle de Cañete. Sociedad Entomológica del Perú (SEP). Programa y resúmenes. XLIV Convención Nacional de Entomología. p 76.

Mukasa, S.B., Rubaihayo, P.R., and Valkonen, J.P.T. 2006. Interactions between a crinivirus, an ipomovirus and a potyvirus in coinfected sweet potato plants. Plant Pathol. 55:458–467.

Ngeve, J.M. and Bouwkamp, J.C. 1991. Effects of sweet potato virus disease (SPVD) on the yield of sweet potato genotypes in Cameroon. Exp. Agric. 27: 221–225.

Ng, J.C.K. and Falk. B.W. 2006. Virus-vector interactions mediating nonpersistent and semipersistent transmission of plant viruses. Annu. Rev. Phytopathol. 47:183–212.

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

OEPP/EPPO. 2004. EPPO Standards PM7/35: Diagnostic protocols for regulated pests. Paris, EPPO Bulletin 34:281–285.

R Development Core Team. 2007. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Schaefers, G.A and Terry, E.R. 1976. Insect transmission of sweet potato disease agents in Nigeria. Phytopathology 66:645–645.

Sheffield, F.M.L. 1958. Virus diseases of sweet potato in East Africa: II. Transmission to alternative hosts. Phytopathology 48:1–6.

Sim, J., Valverde, R.A., and Clark, C.A. 2000. Whitefly transmission of *Sweet potato chlorotic stunt virus*. Plant Dis. 84:1250.

Stansly, P.A. and McKenzie, C.L. 2007. Fourth International Bemisia Workshop International Whitefly Genomics Workshop. *J. Insect Sci.* 8:4 (available online: insectscience.org/8.04).

Tairo, F., Musaka, S.B., Jones, R.A.C., Kullaia, A., Rubaihayo, P.R., and Valkonen, J.P.T. 2005. Unraveling the genetic diversity of the three main viruses involved in Sweet Potato Virus Disease (SPVD), and its practical implications. Mol. Plant Pathol. 6:199–211.

Tamura, K., Dudley, J., Nei, M., Kumar, S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. and Evol. 24:1596–1599.

Trenado, H.P., Lozano, G., Valverde, R.A. and Navas-Castillo, J. 2007. First report of *Sweet potato virus G* and Sweet potato virus 2 infecting sweet potato in Spain. Plant Dis. 91:1687.

Untiveros, M., Fuentes, S., and Salazar, L.F. 2007. Synergistic interaction of Sweet potato chlorotic stunt virus (Crinivirus) with carla-, cucumo-, ipomo- and potyviruses infecting sweet potato. Plant Dis. 91:669–676

Valverde, R.A., Sim, J., and Lotrakul, P. 2004. Whitefly transmission of sweet potato viruses. Virus Res. 100:123–128.

Wintermantel, W.M. 2004. Emergence of greenhouse whitefly (*Trialeurodes vaporariorum*) transmitted crinivirus as threats to vegetables and fruit production in North America. APSnet feature article June 2004. Online publication.

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

Wintermantel, W.M., Wisler, G.C., Anchieta, A.G., Liu, H.-Y., Karasev, A.V., and Tzanetakis, I.E. 2005. The complete nucleotide sequence and genome organization of Tomato chlorosis virus. Arch. Virol. 150:2287–2298.

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

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

Xiong, B., and Kocher, T.D. 1991. Comparison of mitochondrial DNA sequences of seven morphospecies of black flies (Diptera: Simuliidae). Genome 34:306–311.

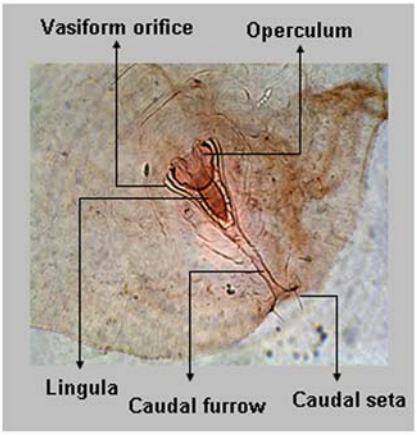
Table 1. Transmission efficiency of *Sweet potato chlorotic stunt virus* (SPCSV) with individual adults of *Bemisia afer* sensu lato and *B. tabaci* biotype B at two temperatures, using sweetpotato (*Ipomoea batatas*) plants cv. Costanero infected with SPCSV (single infection) and with SPCSV and *Sweet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

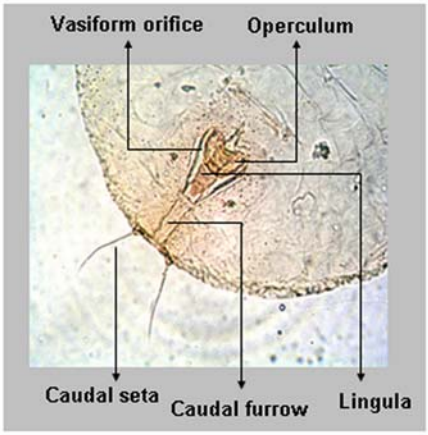
Whitefly species	Virus source	Temperature (°C)													
		20							25						
		Replications				Effi-ciency (%)	STD	SE	Replications				Effi-ciency (%)	STD	SE
		1	2	3	4				1	2	3	4			
B. afer sensu lato	SPCSV	3/23[a]	2/25	2/25	4/25	11.2[b]	0.96	0.24	4/25	2/25	2/25	5/25	13	1.5	0.38
	SPCSV+SPFMV	1/25	3/24	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

Table 2. Statistical analysis by the test of equal or given proportion (n= 98-100) 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)

Comparison of virus source (SPCSV vs. SPCSV+SPFMV)				
	at 20°C		**at 25°C**	
	χ^2	**p-value**	**χ^2**	**p-value**
SPCSV vs. SPCSV+SPFMV for *B. afer* sensu lato	6e-04	0.98	1.33	0.25
SPCSV vs. SPCSV+SPFMV for *B. tabaci* biotype B	1.47	0.23	0.53	0.46
Comparison of vector species (*B. afer* sensu lato vs. *B. tabaci* biotype B)				
	at 20°C		**at 25°C**	
	χ^2	**p-value**	**χ^2**	**p-value**
B. afer vs. *B. tabaci* from SPCSV+SPFMV source	0.19	0.66	0.93	0.33
B. afer vs. *B. tabaci* from SPCSV source	0.62	0.46	0.85	0.36
Comparison of temperatures (20°C vs. 25°C)				
	***B. afer* sensu lato**		***B. tabaci* biotype B**	
	χ^2	**p-value**	**χ^2**	**p-value**
20°C vs. 25°C with SPCSV+SPFMV source	0.26	0.61	0.00	1.00
20°C vs. 25°C with SPCSV source	0.03	0.87	0.00	1.00





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

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

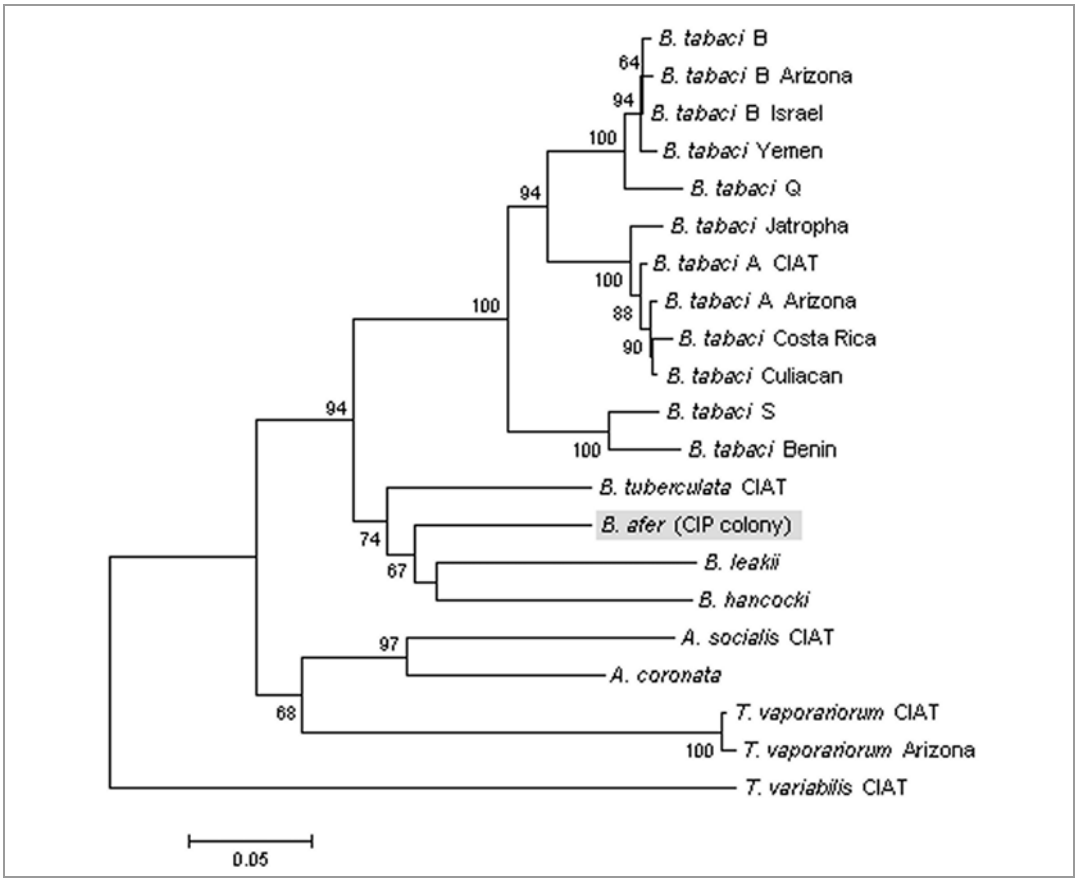


Figure 2. Phylogenetic tree calculated from alignments of nucleotides sequences on the mitochondrial 16S rDNA gene of *B. afer* sensu lato (GenBank accession FJ969473) with other whitefly species. The percentage of bootstrap support out of 2,000 replicates is given at each of the major nodes in the tree. The scale bar indicates Kimura nucleotide distances.