N O T E

A New Haplotype of *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) in Northeastern Mexico[1]

Renato Villegas-Luján, Oscar Sanchez-Flores, Marcela Vasquez[3], Robert Gilbertson[3], Kevin L. Keegan[4], and Sergio R. Sanchez-Peña[2]

Programa de Parasitología Agrícola, Universidad Autónoma Agraria Antonio Narro, Saltillo, Coahuila, Mexico 25315

J. Entomol. Sci. 55(3): 421–426 (July 2020)

Key Words Hemiptera, vector, virus, horticulture, field crops

---

The greenhouse whitefly, *Trialeurodes vaporariorum* Westwood, is an important pest in tropical and subtropical areas and greenhouses worldwide. It is a vector of plant pathogens (Crinivirus and others) including tomato chlorosis virus (Watanabe et al. 2018**,** J. Appl. Entomol 142: 1008–1015). The existence of cryptic species or species complexes in whiteflies has been recognized, particularly in the sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Brown 2010, Pp. 31–67, *In* P. A. Stansly and S. E. Naranjo [eds.], *Bemisia*: Bionomics and Management of a Global Pest. Springer, Berlin; Lee et al. 2013, PLoS One 8(5): p.e63817). These species complexes include morphologically identical populations that are reproductively isolated (i.e., they are valid biological species) and can differ in vectorial efficiency, insecticide resistance, and life history parameters (Alemandri et al. 2015, J. Econ. Entomol. 108: 405–413; Surendran et al. 2019, J. Entomol. Sci. 54: 87–93). Cryptic species can be detected through experimental hybridization or from allele sequences of markers such as cytochrome oxidase 1 (CO1) (Boykin and De Barro 2014, Front. Ecol. Evol. 2: 45; Kapantaidaki et al. 2015, J. Heredity 106: 80–92).

In May 2018 and 2019, conspicuous outbreaks (swarms) of whiteflies were observed in urban and agricultural areas of Saltillo, Coahuila, Mexico, including the Universidad Autónoma Agraria Antonio Narro (UAAAN) (W 101°2′17.98″, N 25°21′15.80″, 1,746 m above sea level). The objectives of the present work were to confirm the identification of this whitefly population, using morphology and CO1 sequencing, and to detect host plants species (i.e., those supporting emergence of adults) among the local, common field vegetation.

---

[1] Received 13 August 2019; accepted for publication 4 November 2019.

[2] Corresponding author (email: sanchezcheco@gmail.com).

[3] Department of Plant Pathology, University of California, Davis, Davis, CA 95616 USA.

[4] Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT 06269 USA.

For morphological examination (Martin 2005, Zootaxa 1098: 1–116), pupae detached from leaves were macerated (cleared) in 40% KOH (20–30 min) in a watch glass. Pupal wax was removed with chloral-phenol (50 g chloral hydrate/50 g phenol) (30 min) at 60°C; soaking in glacial acetic acid (5 min), then treatment in clove oil for at least 2 h. Pupal cases were mounted between slide and coverslip in Canada balsam and examined microscopically at 400–1,000×. Identification followed Martin (2005) and Carapia-Ruiz and Castillo-Gutiérrez (2012, Moscas Blancas: Género *Trialeurodes* Cockerell (Hemiptera: Aleyrodidae) de México, Editorial Académica Española, 104 pp). Specimens are with RVL, SRSP and OSF.

For DNA analysis, adult whiteflies were stored in 96% ethanol. About 25 whiteflies were washed twice with 70% ethanol in water, dried, and then washed with STE buffer (100 mM NaCl, 1 mM EDTA, 10 mM Tris-HCl). Insects were ground in 200 μL of STE buffer, and the suspension centrifuged (5 min, 15,000 × *g*). This whitefly extract was kept at 4°C for 10 min (PCR preparation), and 5 μL was used in each PCR test. Two whitefly samples were used in the reaction: an unknown from Saltillo and a known *B. tabaci* (Q biotype) as a positive control. PCR was performed in 25 μL with 1× Taq polymerase buffer, 5 mM of $MgCl_2$, 1 mM dNTPs, 1 μM of each primer: C1-J-2195-FW (5′-TTGATTTTTTGGTCATCCAGAAGT-3′) and C1-J-2195-RV (5′-TCCAATGCACTAATCTGCCATATTA-3′) (Frohlich et al. 1999, Mol. Ecol. 8: 1683–1691; Marubayashi et al. 2013, J. Appl. Entomol. 137: 113–121; Simon et al. 1994, Ann. Entomol. Soc. Am. 87: 651–701), and 0.5 U of Taq polymerase (Denville, Montreal, Canada). This primer pair was designed for PCR of a *B. tabaci* CO1 fragment. It can be used similarly in different species of Aleyrodidae (M.V., pers. obs.). PCR parameters were: 94°C (5 min), 35 cycles of 94°C (30 s), annealing (45°C, 45 s), and extension (1 min, 72°C), followed by extension (72°C, 10 min). PCR products were examined by electrophoresis (1% agarose gels). The expected (near 800 base pair) DNA fragment amplified from the unknown Saltillo sample was purified and sequenced at Quintara Bioscience (San Francisco, CA, USA) with primers C1-J-2195-FW and C1-J-2195-RV. Sequences were compared (BLAST; Basic Local Alignment Search Tool) with GenBank accessions and deposited in that database.

For identification of whitefly host plants in the field, we collected leaves harboring nymphs or pupal cases (Table 1). Empty pupal cases with a characteristic T-shaped slit indicate development and emergence of adults (S.-F., pers. obs.). Additionally, development was verified in the laboratory whereby field-collected leaves harboring eggs or nymphs were incubated at room temperature in moist chambers (plastic containers with a moistened cotton ball and small ventilation holes). Under these conditions, if the plant is a suitable host, at least some whiteflies are usually able to develop to the adult stage before plants decompose. Pupal cases were identified.

The following morphological traits, among others, indicated that this outbreak population belonged in *T. vaporariorum*: lobed head of the lingula; submargin often presenting a row of papillae that can be visible or reduced; abdomen with segments three and four with two pairs of subdorsal pores and one pair in each of the four posterior segments; papillae well or not well developed near most pores; subcutaneous papillae 8–12 μm long and 12–16 μm wide (Carapia-Ruiz and Castillo-Gutiérrez 2012; Martin 1987, Int. J. Pest Manag. 33: 298–322;)

The CO1 sequence obtained (accessed as GenBank MK990565) had 85–86% similarity (the highest) to sequences reported by Kapantaidaki et al. (2015) in a worldwide analysis of *T. vaporariorum*. They found only two very similar haplotypes, a "Northern hemisphere" and a worldwide marker, differing only 0.15% among them. Similarly, a worldwide analysis found very low levels of CO1 variability (i.e., single nucleotide polymorphisms) in this insect (Prijović et al. 2014, Bull. Entomol. Res. 104: 357–366). These authors proposed three *T. vaporariorum* haplotypes. This worldwide uniformity might indicate recent colonization events and/or low population discrimination value for the CO1 marker.

By comparison, the sequence reported in the present work is highly different (14–15% divergence) from *T. vaporariorum* sequences in GenBank, such as those (i.e., KJ475452) reported by Kapantaidaki et al. (2015). The CO1 sequence obtained had also 85.6% similarity to numerous sequences from Costa Rica. It was 81.29–81.51% similar to analogous sequences from the sweetpotato whitefly (*B. tabaci*) (Guevara-Coto et al. 2011, Eur. J. Plant Pathol. 131: 167–170). By comparison, a threshold of 2.2% was considered adequate for separation of valid species in Hemiptera: Heteroptera. This level is reportedly 2% for Ephemeroptera, Lepidoptera, Plecoptera, and Trichoptera (Lin et al. 2015, PLoS One 10(9): p.e0138993, and references therein). Lin et al. (2015) suggested an unusually high level of 4–5% sequence divergence as appropriate in practice to separate species of *Tanytarsus* midges (Diptera: Chironomidae).

The observations on plants found to support whitefly development (adult emergence) are summarized in Table 1. The field plant host range of these populations was similar to that reported for *T. vaporariorum*, but expanded (Table 1). They colonized usual hosts such as dry beans, *Phaseolus vulgaris* L. (Fabaceae); potato, *Solanum tuberosum* L. (Solanaceae); dandelions, *Taraxacum officinale* (L.) (Asteraceae); and others. However, they also colonized and reproduced on unusual hosts (for local *T. vaporariorum*) such as pecan rootstock (*Carya* sp.) and cultivated (grafted) pecan, *Carya illinoinensis* (Wangenheim) Koch (Juglandaceae); hackberry, *Celtis laevigata* Willdenow; ash, *Fraxinus berlandieriana* De Candolle (Oleaceae); and elm, *Ulmus* sp. (Ulmaceae); and on different herbaceous weed hosts. In our open field experience from decades, most plants in Table 1 are not colonized to any noticeable extent by local, "usual" *T. vaporariorum* (i.e., populations previous to the 2018 population outbreak described herein). Among the trees listed in the present work, Dooley (2011, https://keys.lucidcentral.org/keys/v3/whitefly/key/Aleyrodid%20Pupal%20Key%20to%20the%20Genera/Media/Html/Host_Pests.htm. 12 August 2019) lists the following as hosts: pecan, *C. illinoinensis* (as *Carya pecan*), ash (*Fraxinus* sp.), and hackberry (*Celtis occidentalis* L.). Elms (*Ulmus* spp.) are not listed as hosts (see Table 1). Santa Maria weed, *Parthenium hysterophorus* L. is listed as host (Dooley 2011). This was unexpected because *P. hysterophorus* is a weed target of international biological control projects, and intensive surveys of *P. hysterophorus* herbivores in central and northern Mexico and Texas did not detect *T. vaporariorum*, only *Trialeurodes abutilonea* Haldeman (McClay et al. 1995, Environ. Entomol. 24: 796–809). In pecan, most adults landed and oviposited on pecan rootstock suckers as opposed to suckers or tender shoots of grafted pecan. Rootstock foliage harbored more than 10 times any given whitefly stage compared to the graft foliage. To the best of our knowledge, *T. vaporariorum* is not reported as a pest of, or as colonizing, pecans.

Table 1. Plant host use by *T. vaporariorum*, Saltillo. Plant material collected 23 March–14 April of 2018 and 2019. Individual species were collected repeatedly in this time period.[A]

Host Plant (Common Name in English and Spanish and Scientific Name)	Pupal Cases (i.e., Adult Emergence) Present in the Field	Eggs and Nymphs Present in the Field	Adults Emerged From Eggs or Nymphs on Plant, in the Laboratory
*Hackberry/Palo blanco *Celtis laevigata* +, −−	+	+	+
*Cultivated (grafted) Pecan/Nogal *Carya illinoinensis* +, −−	+	+	d
*Pecan rootstock/ portainjerto de Nogal, *Carya* sp. +, −−	+	+	d
Pigweed/Amaranto o Quelite, unident. Chenopodiaceae −, −−	+	+	d
Fleabane, *Erigeron bonariensis* L. −, −−	+	+	+
Fetid Marigold, *Dyssodia papposa* (Ventenat) Hitchcock) −, −−	+	+	+
*Ash/Fresno, *Fraxinus berlandieriana* De Candolle +, −−	+	+	All insects (pupae, $n =$ 23) parasitized by Aphelinidae; full development seemed likely
Prairie Verbena/ Verbena *Glandularia bipinnatifida* (Nuttall) Nuttall −, −−	−	+	d
*Common Mallow/ Malva *Malva sylvestris* L. +, −−	+	+	d

Table 1. Continued.

Host Plant (Common Name in English and Spanish and Scientific Name)	Pupal Cases (i.e., Adult Emergence) Present in the Field	Eggs and Nymphs Present in the Field	Adults Emerged From Eggs or Nymphs on Plant, in the Laboratory
Broomweed, *Malvastrum* (L.) +, ++	+	+	d
*Santa-Maria weed/ amargoso, *Parthenium hysterophorus* +, −−	+	+	+
Beans/frijol o judía (*Phaseolus vulgaris*) +, ++	+	+	+
Curly dock/lengua de vaca *Rumex crispus* L. −, −−	+	+	d
Potato/papa o patata (*Solanum tuberosum*) +, ++ (on *Solanum* sp.)	+	+	+
Goldenrod/vara de oro, *Solidago velutina* De Candolle. +, ++ (on *Solidago* sp.)	+	+	+
Sow thistle/cardunch a o cerraja (*Sonchus oleraceus* L.) +, ++	+	+	+
Dandelions/Diente de león (*Taraxacum officinale*) +, −−	+	+	+
*Elm/Olmo (*Ulmus* sp.) −, −−	+	+	+

[A] Abbreviations: + = present; − = Not present; d = plant material decomposed, insect mortality ensued. Plus (+) and minus (–) signs next to each plant's scientific name indicate the following: +, listed or −, not listed as host in Dooley (2011); ++, listed, or −−, not listed as host in Evans (2007).

* New or unusual host for *T. vaporariorum*.

An unidentified species of creeping Chenopodiaceae was extremely attractive to these whiteflies, which covered both upper and undersides of leaves with eggs in the field. This unusual phenomenon was observed in other herbaceous hosts as well. In the host plant list by Evans (2007, USDA/Animal Plant Health Inspection Service [APHIS], Document version 70611), the author indicates that *T. vaporariorum* is highly polyphagous and that "it would not be surprising to find this species on almost any terrestrial plant." However, after decades of observations in this locality (Saltillo) and other sites, we have found, in fact, many plants that are not exploited as hosts by local, "conventional" (pre-2018 outbreak) *T. vaporariorum* (i.e., orange, *Citrus sinensis* (L.) Osbeck, pecan, hackberry, ash, elm). These whiteflies were very conspicuous and abundant in open areas even under summer dry periods lasting several days with relative humidity around 20% (another unusual trait for *T. vaporariorum*). The presence of a widely diverging haplotype in this Mexican *T. vaporariorum* outbreak population indicates that this widespread whitefly morphospecies might include cryptic species.