N O T E

Natural Enemies Associated with *Brevipalpus* sp. (Acari: Tenuipalpidae), Vector of Citrus Leprosis[1]

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Citrus leprosis is a viral disease that affects the leaves, branches, and fruits of mainly orange and mandarin trees (*Citrus* spp.) (León et al. 2017, Rev. Colomb. Entomol. 43: 215–222), crippling the production of fruits and causing tree death (Rodrigues 2000, Manejo Integrado de Plagas [Costa Rica] 60: 61–65). It is considered one of the most destructive citrus diseases in America (Rodrígues et al. 2003, Exp. Appl. Acarol. 30[1–3]: 181–202). Economic losses caused by it can amount to US$ 60 million a year, and the associated expenses for vector control approach US$ 75 million (Bastianel et al. 2006, Phytopathol. 32[3]: 211–220). Although orange and mandarin trees are reported to be the main hosts of this disease, in Mexico it has been detected in acid citrus fruits such as Persian lemon, *Citrus latifolia* Tanaka, and Mexican lemon, *C. aurantifolia* (Christm.) (Swingle) (Alanis-Martínez et al. 2013, Congr. Nac. Soc. Mexicana de Fitopatol. Pg. 8.).

In Mexico, citrus leprosis was reported for the first time in 2004 in the state of Chiapas. By 2018, it was recorded in 11 states of the Mexican Republic (CAB International 2018, https://www.cabi.org/isc/datasheet/13449, accessed 31 January 2019), including Jalisco (SENASICA, 2019 https://www.gob.mx/cms/uploads/attachment/file/463428/Ficha_T_cnica_Leprosis_de_los_c_tricos.pdf, accessed 22 August 2019), which borders the state of Colima where lemon production is the fourth largest among all states in Mexico (SIAP 2019, http://infosiap.siap.gob.mx:8080/agricola_siap_gobmx/AvanceNacionalCultivo.do, accessed 31 August 2019). Occurrence of the disease has not yet been officially reported in Colima.

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Citrus leprosis spreads to new areas through infected but asymptomatic citrus seedlings or through virus-carrying mites of the genus *Brevipalpus* (Acari: Tenuipalpidae) (NAPPO 2014, http://nappo.org/application/files/1415/8323/0051/ST-06-Citrus-Leprosis_26-11-2015-e.pdf; accessed 27 August 2019). These mites are considered the most important group of the family Tenuipalpidae due to their ability to cause direct and indirect damage. They cause direct damage to host plants when feeding by inducing chlorotic or necrotic lesions in the feeding sites (Kitajima et al. 2004, Summa Phytopathol. 30: 68), but most of the damage is caused indirectly by transmitting the virus that causes citrus leprosis (Solano et al. 2008, Agron. Colomb. 26: 399–410).

Of the 10 species of the genus *Brevipalpus* currently known to be associated with citrus plants, *B. californicus* (Banks), *B. obovatus* (Donnadieu), and *B. yothersi* (Baker) (=*B. phoenicis* Geijkes) have been reported as vectors of citrus leprosis (González-Arias et al. 2009, Fitosanidad 13: 79–81). However, *Brevipalpus* is widely spread in Mexico and is reported in 18 states (Santillan-Galicia et al. 2018, Simpósio Brasileiro de Acarología. http://www.infobibos.com/anals/sibac/6/resumos/ResumoClac3Sibac6_0091.pdf; last accessed 27 August 2019).

Citrus leprosis is a very serious disease that can spread rapidly and, once established, has no cure. Control tactics are eliminating sources of inoculum by cutting and destroying affected trees and using miticides to control the mite vectors (Chagas 2000, Compendium of Citrus Diseases, 2nd ed., Am. Phytopathol. Soc. Press, St. Paul, MN, USA). However, the intensive use of agrochemicals has several deleterious impacts, including development of pest resistance and elevation of secondary pests that had no previous importance. Moreover, agrochemicals have negative effects on human and environmental health (Ferrer 2003, Ann. Sis. San Navarra 26: 155–171). We, therefore, undertook this study with the aim of surveying for and identifying natural enemies of *Brevipalpus* sp. and conducting comparative tests of the potential predatory or parasitism capacity for consideration as candidates for integrated management of the citrus leprosis vector.

Surveys for natural enemies of *Brevipalpus* sp. were conducted in orange trees growing in six locations in the state of Colima. Those were the municipal capital of Comala (19°19′23″N; 103°45′30″W), El Galaje in Ixtlahuacán (18°51′31″N; 103°38′08″W), Colima City (19°13′53″N; 103°43′52″W), Tecomán City (18°54′49″N; 103°52′33″W), Tepames in Colima (19°05′38″N; 103°37′21″W), and Las Golondrinas in Colima (19°08′23″N; 103°46′20″W). Sampling was conducted every 15 d for 14 mo, starting on 24 April 2018 and ending on 4 June 2019. On each sampling date and at each site, 40 leaves and 5 fruits were collected from 5 randomly selected trees. The circumference of each tree was measured at a height of approximately 1.5 m above ground surface. At each site, the presence of *Brevipalpus* spp. was verified using a hand-held 10× magnifier. Collection data were recorded, and samples were appropriately labeled and transported to the laboratory at the Department of Entomophagous Insects of the National Center for Biological Control Reference (DIE-CNRCB) in the City of Tecomán, Colima, Mexico. Natural enemies were separated and identified using dichotomous keys of Gordon (1985, Entomol. Soc. 93: 1–912) and Gordon and Vandenberg (1991, Proc. Entomol. Soc. Washington 93: 845–867) for coccinellids and Tauber et al. (2000, Ann Entomol. Soc. Am. 93: 1195–1221) for neuropterans. Predatory mites were identified through extraction of genomic DNA using a nondestructive method with

DNeasy® Blood and Tissue Kit (Qiagen®, CA, USA). Species diversity in the sampled areas was determined according to the Shannon and Simpson 1-D indices. *Brevipalpus* sp. were identified using specific primers for *Brevipalpus* (Laboratory of Molecular Biology of the CNRCB). The identification of the mites was corroborated by Dr. Ma. Teresa Santillán Galicia (Phytosanitary Institute of Colegio de Postgraduados, Montecillos, Texcoco, México). Specimens were deposited in the Entomophagous Insect Collection of the National Reference Center for Biological Control.

Natural enemies deemed as potential predators of mites were evaluated for predation of the mites. The physical size of *Brevipalpus* sp. (0.29 mm in length) (Nunes 2020, https://www.nappo.org/application/files/8315/8771/0488/4_Maria_Andreia_Nunes_Etiologia_Historia_Leprosis.pdf; accessed 16 January 2020) precludes efforts to observe natural enemy activity against the mites in the field. Thus, these tests were conducted in the laboratory of DIE-CNRCB using Petri dishes (5-cm diameter) as arenas. A 5-cm diameter orange leaf disc was placed on a moistened 5-cm diameter sterile filter paper in each Petri dish. Fifty individuals of *Brevipalpus* sp. were placed on each leaf disc using a fine paint brush and, 1 h later, a single individual of a potential predator collected in the field was introduced. Activity of *Brevipalpus* sp. and the potential predator was recorded for 24 h using a stereoscopic microscope.

Predatory capacity of individual natural enemies also was estimated by the previously described test. Again, 50 *Brevipalpus* sp. were on the leaf disc in each Petri dish and, 1 h later, one predator specimen was introduced into each experimental arena. These natural enemies had been starved for 12 h before introduction. Adults and each of the instars were evaluated when required. The first instar was evaluated 24 h after emerging from the egg, and the ensuing instars were evaluated 24 h after molting from the immediately preceding immature stage. The number of *Brevipalpus* sp. individuals consumed in a period of 5 h was counted. The individuals consumed by the predator were not replaced during the experiment. Ten repetitions were performed in an environmental chamber maintained at 25 $\pm$ 2°C, 70 $\pm$ 10% relative humidity (RH), and on a 14:10 h L:D photoperiod. Data were analyzed with ANOVA, and treatment means were separated Tukey's multiple comparison test ($\alpha = 0.05$) (SAS® 2008, SAS Institute, Cary, NC, USA).

Eight natural enemies associated with the presence of *Brevipalpus* sp. were identified. Those were *Stethorus* sp. (Weise), *Diomus terminatus* (Say), *Azya orbigera* (Mulsant) (Coleoptera: Coccinellidae), *Ceraeochrysa cincta* (Schneider), *C. valida* (Banks) (Neuroptera: Chrysopidae), *Amblyseius largoensis* (Muma), *Typhlodromus occidentalis* (Nesbitt) (Acari: Phytoseiidae), and an unidentified mite of the family Cunaxidae (Acari: Trombidiformes) (Table 1).

Shannon (1.139) and Simpson 1-D (0.6654) indices, as well as the effective number of species (3.739), indicated that Tepames had the highest diversity of species associated with the presence of *Brevipalpus* sp. Table 1 shows that *C. cincta* was the predator most frequently found in the sampled areas, whereas species of family Phytoseiidae were present in larger numbers.

Mites of the genus *Brevipalpus* were identified as *B. yothersi* and *B. californicus*. *Brevipalpus yothersi,* considered synonymous with *B. phoenicis* (Pritchard and Baker 1952, Univ. California Publ. Entomol. 9: 1–94), is one of the most important

Table 1. Number of natural enemies collected in associated with the presence of *Brevipalpus* sp. in six locations in the state of Colima, April 2018 to June 2019.

Species	El Galaje	Comala	Colima City	Tecomán City	Las Golondrinas	Tepames	Total
C. cincta	10	23	21	8	72	57	191
C. valida	9	3	0	1	7	12	32
A. orbigera	0	0	11	0	0	0	11
A. largoensis/ T. occidentalis	0	0	0	0	274	97	371
Cunaxidae	0	0	0	0	0	3	3
Stethorus sp.	0	0	0	0	5	26	26
Diomus sp.	0	0	0	0	0	6	6

flat mites due to the economic losses they cause in crop damage (Novelli et al. 2016, Proc. 8th Symp. EURAAC. Valencia, Spain, 11–15 July 2016). It is considered the most widespread and effective vector in the world when transmitting cytoplasmic leprosis viruses (citrus leprosis virus C, citrus leprosis virus C2, and Hibiscus green spot virus 2) (Leon et al. 2006, Plant Dis. Note 90: 682). *Brevipalpus californicus* also has been reported as an infrequent vector of these viruses (Rodrigues and Childers 2013, Exp. Appl. Acarol. 59: 165–175).

In Mexico, Santillán -Galicia et al. (2018) reported species of the genus *Brevipalpus* in 18 states. Rosas-Acevedo and Sampedro-Rosas (2006, Rev. Mex. Biodivers. 77: 7–16) reported the presence of *B. phoenicis* in the town of El Paraíso, in the Municipality of Tecomán, Colima. However, to date there is no record of *B. californicus* in the state of Colima. Our collection, thus, represents the first report of this phytoseiid in the study area.

The consumption tests showed that *Brevipalpus* sp. mites were consumed by the chrysopids *C. cincta* and *C. validat*, the phytoseiids *A. largoensis* and *T. occidentalis*, and Cunaxidae specimens. In contrast, the coccinellids *Stethorus* sp., *D. terminates*, and *A. orbigera* did not feed on either *B. yothersi* or *B. californicus*. These observations differ from those reported in the literature, in which species of the genera *Stethorus* sp., *Diomus* spp., and *Azia* sp. are reported as predators of phytophagous mites (Biddinger et al. 2009, Biol. Contr. 51: 268–283). Chazeau (1985, Their Biology, Natural Enemies, and Control, vol. 1B. Elsevier, Amsterdam, pp. 211–246) reported that the larvae and adults of *Stethorus* sp. are specialist predators of mites belonging to the families Tetranychidae and Tenuipalpidae. Guanilo and Martínez (2007) (Ecología Aplicada 5[1, 2]: 117–129) have associated *Stethorus* species with mites of the Tetranychidae family, whereas León and Kondo (2017) (Corpoica Cienc Tecnol Agropecuaria, Mosquera (Colombia), 184 p.) reported that *Stethorus* species are important for citrus farming because they feed on the eggs of phytophagous mites. Similarly, Aguilera's (1987, Rev. Chil. Entomol.

15: 33–36) assessment of pest biological control in the Republic of Chile indicates that beetles belonging to the genus *Stethorus* sp. are moderate predators of several species of mites, including *B. chilensis* (Baker) (Prostigmata: Tenuipalpidae). Furthermore, Biddinger et al. (2009, Biol. Contr. 51: 268–283) listed several species of mites as prey for *Stethorus* sp.; however, no *Brevipalpus* species were included in their list. There also are reports of *Diomus seminulus* Mulsant and of *A. orbigera* consuming mites of the family Tetranychidae (*Tetranychus* sp.) (Acari: Tetranychidae) (León and Kondo 2017), but there were no reports of these predators feeding on *Brevipalpus* species. Available literature and observations reported in this study suggest that these coccinellids feed on mites of the Tetranychidae and Tenuipalpidae families but not on species belonging to the genus *Brevipalpus*, in particular *B. californicus* and *B. yothersi*. However, further studies are needed to corroborate this assumption.

With regard to the chrysopids evaluated in our study, several authors indicate that they are generalist predators with their diets including phytophagous mites (Carvalho and Souza 2009, Revista Caatinga 27: 177–182). Carvalho and Souza (2009) mentioned the potential of chrysopids as natural enemies of pest mites, including *B. phoenicis*. Similarly, without naming the mite species, Tauber and De León (2001) (Ann. Entomol. Soc. Am. 94: 197–209) reported *C. cincta* feeding on mites found on citrus fruit trees (*Citrus* spp.) in the state of Colima, Mexico. Furthermore, Contreras-Bermúdez et al. (2017) (J. Entomol. Sci. 52: 460–462) reported *C. cincta* and *C. valida* feeding on the red palm mite, *Raoiella indica* (Hirst) (Acari: Tenuipalpidae). Given the results presented herein and the information gleaned from available scientific literature, in which there is no mention of *Brevipalpus* species serving as prey to these chrysopids, it can be inferred that our study is the first report of predation by *C. cincta* and *C. valida* of *B. californicus* and *B. yothersi*.

Guarin (2003, Antioquia [Colombia] Editorial: Gráficas Madrigal) reported that third-instars are the most voracious of chrysopid developmental stages in predatory activities. However, our results showed that first instars (L1) of *C. cincta* and *C. valida* consumed significantly more mites than other developmental stages ($F =$ 14.78; $P > 0.0001$) (Table 2). Our findings corroborated those of Silva et al. (2006, Coffee Sciences, Lavras 1: 50–54) who reported that first-instar *C. externa* (Hagen) (Neuroptera: Chrysopidae) demonstrated a higher predatory capacity compared with second and third instars fed *B. phoenicis*. Those results may be explained by the hypothesis raised by Snadness and MacMurtry (1970, Can. Entomol. 102: 692–704) who suggested that the ratio between the size of the predator and its prey is an important factor in the efficiency of the predatory process.

The mites *A. largoensis* and *T. occidentalis* consumed a mean (±SD) of 10.8 ± 1.3 and 8.3 ± 1.0 *Brevipalpus* sp. individuals each, respectively (Table 2). There is little information on the predatory capacity of these mites against *Brevipalpus* sp. Badii et al. (2010, Intern. J. Good Consc. 5: 270–302) stated that the vast majority of phytoseids feed on species of the Tetranychidae family, while Tenuipalpidae species are not frequently preyed upon. However, Haramoto (1968, Hawaii Agric. Exp. Sta. Tech. Bull. 68: 1–63) and Hastie et al. (2010, Revista Protección Vegetal 25: 17–25) reported *A. largoensis* as a predator of *B. phoenicis*, and Kamburov (1971, J. Econ. Entomol. 64: 643–648) and Serra-Galvão et al. (2008, Ciência Rural, Santa Maria 38: 1817–1823) reported that *A. largoensis* successfully

Table 2. Mean (±SD) number of *Brevipalpus* sp. mites consumed by *C. valida*, *C. cincta*, *A. largoensis*, and *T. occidentalis* in laboratory arenas in a period of 5 h.

Species	Stage and/or Instar	Mites Consumed*
C. valida	L1	21.4 ± 2.2 a
	L2	7.8 ± 1.0 bc
	L3	1.9 ± 0.6 c
C. cincta	L1	23.2 ± 3.3 a
	L2	11.0 ± 2.3 b
	L3	7.2 ± 1.5 bc
A. largoensis	Adult	10.8 ± 1.3 b
T. occidentalis	Adult	8.3 ± 1.0 bc

* Means followed by the same lowercase letter are statistically equal (Tukey, $\alpha = 0.05$).

reproduced when feeding on *B. phoenicis*. Likewise, Ragusa and Vargas (2002, Phytophaga 12: 129–139) reported species belonging to the genera *Amblyseius* and *Typhlodromus* associated with *B. chilensis*.

Rodríguez et al. (2010, Revista Protección Vegetal 25: 26–30) and Posos-Ponce et al. (2019, Entomol. Mex. 6: 8–13) noted the predatory capacity of *A. largoensis* against the red palm mite, indicating that it is capable of consuming a mean (±SD) of 1.30 ± 0.97 and 5.71 ± 1.23 individuals in 30 min, respectively. Moreover, Pruszynski and Cone (1973, Ann. Entomol. Soc. Am. 66: 47–51) and Biological Services (2015, https://biologicalservices.com.au/products/occidentalis-31.html, accessed 15 January 2020) stated that *T. occidentalis* are capable of consuming 1.9 to 3.6 and 5 to 15 adults of *T. urticae* in 24 h, respectively.

Because they are generalist predators and can be found in various crops, Díaz-Tejeda et al. (2010, Revista CitriFrut 27: 54–63) indicated that the species belonging to the genus *Amblyseius* have potential as natural enemies of phytophagous mites. Similarly, Badii et al. (2010) reported that *T. occidentalis* could be used to control species belonging to the genus *Tetranychus*; however, its ability to control red spider mite species belonging to other genera is limited.

One mite from the family Cunaxidae (n.i.) was observed feeding on *Brevipalpus* specimens in our consumption tests. The predatory capacity test was not conducted because this mite did not appear again at the sampling sites. In a similar study, González et al. (1985, Agronomía Costarricense 9: 205–2011) reported that they found only one individual of the family Cunaxidae during an entire year associated with stationary mites. However, Solano and Aguilar (2010, (http://www.infobibos.com/anais/sibac/6/resumos/ResumoClac3Sibac6_0092.pdf, accessed 30 September 2019) reported the presence of species from this family cohabiting with several mites of the genus *Brevipalpus*. Lahiri et al. (2004, Proc. Zool. Soc. (Calcutta), 57: 47–52) and Castro and Morales (2010, Exp. Appl. Acarol. 50: 133–139) reported Cunaxidae species feeding on phytophagous mites.

Although species belonging to the family Cunaxidae have been observed feeding on different agricultural pests, Smiley (1992) (Indira Publ. House, USA, 356 p.) and Skvarla et al. (2014, ZooKeys 418: 1–103) consider them as opportunistic predators that can be in a certain environment searching for food, such as springtails or other arthropods, and feed opportunistically on phytophagous mites, as may have occurred in the consumption tests we conducted. Similarly, Nucifora and Vacante (1986, Proc. Experts' Meeting, Acireale (Italy), March 1985. A. A. Balkema, Rotterdam, 369–372) reported that Cunaxidae species are auxiliary predators that can be beneficial for crops, but are not the main predators of crop pests. It would be necessary to conduct studies on their predatory capacity and prey preference to determine how feasible it would be to use them in biological control programs against agricultural pests.

The results obtained herein indicate that *C. cincta*, *C. valida*, *A. largoensis,* and *T. occidentalis* are capable of feeding on *Brevipalpus* sp. However, we must consider that the artificial no-choice situation in which the experiment was conducted, forced the starved predators to feed on the only available prey in the experimental arena. It is, thus, recommended to develop studies on prey preference as well as biological aspects (e.g., predatory capacity, life tables, fertility tables, etc.) to further identify the potential of these predators in the integrated management of *Brevipalpus* sp.