Zoosymposia 6: 39–44 (2011)
www.mapress.com/zoosymposia/

ISSN 1178-9905 (print edition)
ZOOSYMPOSIA
ISSN 1178-9913 (online edition)

Asymmetry in the number of solenidia on tarsi II of *Brevipalpus* (Acari: Tenuipalpidae) populations from Argentina*

ELLIOT W. KITAJIMA[1], ALINE D. TASSI[1], VALDENICE M. NOVELLI[2], SARA CACERES[3], ALCIDES AGUIRRE[3], NORMA COSTA[4] & GILBERTO J. DE MORAES[5]
[1]*Depto. Fitopatologia & Nematologia, ESALQ/USP, 13418-900 Piracicaba, SP, Brazil;*
E-mail: ewkitaji@esalq.usp.br
[2]*Centro APTA Citros Sylvio Moreira-IAC, CP 4, 13490-970 Cordeirópolis, SP, Brazil;*
[3]*EEA Bella Vista, INTA, CC 5, 3432 Bella Vista, Corrientes, Argentina;*
[4]*EEA Concordia, CC 34, 3200 Concordia, Argentina;*
[5]*Depto. Entomologia e Acarologia, ESALQ/USP, 13418-900 Piracicaba, SP, Brazil.*

* *In:* Moraes, G.J. de & Proctor, H. (eds) Acarology XIII: Proceedings of the International Congress. Zoosymposia, 6, 1–304.

Abstract

To verify the prevailing *Brevipalpus* species in citrus orchards affected by leprosis in Argentina, specimens of this genus were collected mainly from 'Valencia' and 'Hamlin' varieties of sweet orange plants from experimental and commercial plantations at Bella Vista, Corrientes Province, Concordia, Entre Rios Province and Saens Peña, Chaco Province. Examinations under light microscopy and scanning electron microscopy were carried out to identify the species based on external morphological characteristics. A mixture of typical *Brevipalpus obovatus* Donnadieu and *Brevipalpus phoenicis* (Geijskes) was found, with prevalence of the former (43.7 versus 27.2%) in the 327 adult females examined. However, 29% of the total number of females examined were atypical, having one solenidion (ω") on one of tarsi II and two (ω' and ω") on the other, either left or right. To investigate whether this variation was also common elsewhere, several *Brevipalpus* populations of the *obovatus* species-group from Chile (*Brevipalpus chilensis* Baker), Brazil (*B. obovatus* and *B. phoenicis*), Costa Rica and Paraguay (*B. phoenicis* in both countries) were examined. Asymmetric numbers of tarsus II solenidia were found in 11 of the 1,993 examined adult female specimens of these populations. The results suggested a relation between the high proportion of asymmetric mites in Argentina and the occurrence of a mixed population of both *B. obovatus* and *B. phoenicis*; however, present knowledge about the apparently parthenogenetic system of reproduction of these species does not support the hypothesis that asymmetric mites could be the hybrids of those species. Thus, the factor leading to high proportions of asymmetric *Brevipalpus* in Argentina remains unknown.

Key words: *Brevipalpus obovatus*, *Brevipalpus phoenicis*, scanning electron microscopy, light microscopy.

Introduction

Some American *Brevipalpus* mites of the *obovatus* species group (Gonzalez, 1975) are notorious for their ability to transmit plant viruses and for their peculiar biology (Childers & Derrick, 2003). These mites are haploid and reproduce by thelytokous parthenogenesis (Childers *et al*., 2003), although a few males are commonly found in natural populations. The latter can copulate but apparently are not able to inseminate females (Pijnaker *et al*., 1981). Weeks *et al*. (2001) demonstrated that the haploid individuals were feminized because of the presence of a symbiont bacterium, a unique case of this sort among mites. At least three tenuipalpid species, namely *B. californicus* Banks, *B. obovatus* Donnadieu and *B. phoenicis* (Geijskes), are known to be involved in the transmission of plant viruses (Childers *et al*., 2003; Kitajima *et al*., 2003).

The morphological characters most commonly used to separate those three species are the number of the dorsolateral hysterosomal setae and the presence or absence of solenidion ω' on the tarsus of leg II of adult females. *Brevipalpus obovatus* and *B. phoenicis* have five (d_3, e_3, f_3, h_1 and

Accepted by H. Proctor: 30 Jul. 2011; Published: 20 Dec. 2011

h_2) and *B. californicus* has six (d_3, e_3, f_2, f_3, h_1 and h_2) pairs of dorsolateral idiosomal setae. In *B. californicus* and *B. phoenicis*, both solenidia ω' and ω"are present, while in *B. obovatus*, only ω" is present (Welbourn *et al*., 2003).

First described in Florida in the beginning of the 20th century, citrus leprosis was first reported in Argentina in the 1920's as "lepra explosiva" (Spegazzini, 1920; Zeman, 1932; Marchionatto, 1935). Also in Argentina, a *Brevipalpus* species was first demonstrated to be involved in the transmission of the causal agent of that disease (Frezzi, 1940; Vergani, 1945). The mite was then reported as *Tenuipalpus pseudocuneatus* Blanch, a junior synonym of *B. obovatus*, as summarized by Mesa *et al*. (2009). Subsequent studies of citrus leprosis, found in most of the South and Central American countries as well as in Mexico, consistently indicated the vector to be *B. phoenicis*, as demonstrated in Brazil for the first time by Musumeci & Rossetti (1963).

After the initial works by Frezzi (1940) and Vergani (1945), no additional information regarding the status of the vector of citrus leprosis in Argentina was published. The present article refers to a recent survey of *Brevipalpus* mites associated with citrus leprosis in Argentina, where an unusual morphological variation of *Brevipalpus* mites was found, and elsewhere in South America and Costa Rica.

Materials and Methods

Samples from Argentina were mainly collected from plants of the 'Valencia' or 'Hamlin' varieties of sweet orange [*Citrus sinensis* (L.) Osbeck] from experimental plots of the Estación Experimental Agropecuaria of the Instituto Nacional de Tecnologia Agropecuaria at Bella Vista, Corrientes Province (EEABV) and at Concordia, Entre Rios Province (EEAC), as well as from commercial orchards at Colonia Tres de Abril, in Bella Vista, and Saens Peña, Chaco Province. A few other samples were collected from 'Nova' mandarin (*Citrus reticulata* Blanco) and *Rhododendron* sp. plants from EEABV. Other *Brevipalpus* samples were collected from the following sources: colonies maintained on sweet orange fruits at the Departamento de Fitopatologia e Nematologia, ESALQ, Piracicaba, State of São Paulo, Brazil and at the Centro APTA Citros Sylvio Moreira, Cordeirópolis, State of São Paulo; *Solanum violaefolium* Schott from Piracicaba; sweet orange plants from Costa Rica and Paraguay; and *Ligustrum lucidum* W.T. Aiton from Santiago, Metropolitan region and Mostazal, O'Higgins (VI[a]) region , in Chile.

Leaf and twig samples were collected in plastic bags, taken to a laboratory in the country where they were collected and fixed in 70% ethanol for later processing for light and scanning microscopy at ESALQ.

For scanning electron microscopy examination, mites were transferred to 95% ethanol for 10 min and then air dried on the surface of a filter paper. Dried adult mites were mounted on the surface of a double faced, carbon containing, adhesive tape glued to the top of an aluminum stub. These mites were positioned in such a way as to make possible the appropriate observation of the dorsodistal part of tarsus of leg II. Samples were gold coated in a Baltec SCD 050 sputter coater and examined in a LEO 435 VP scanning electron microscope (SEM). Some of the specimens from Argentina were mounted in Hoyer´s medium on microscope slides for examination under a light microscope (LM).

Results

On sweet orange plants of EEABV and EEAC as well as of the commercial orchards at Colonia Tres de Abril and Saens Peña, a mixture of the typical *B. obovatus* and *B. phoenicis* was found (Table 1),

TABLE 1. *Brevipalpus* species found in in several South American countries and in Costa Rica, with information on the respective numbers of solenidia on the tarsi of leg II of adult females, as observed by scanning electron microscopy.

Country/Locality	Plant species	Distribution of solenidia		
		2:2	1:1	2:1/1:2
	Mixed infestation by *B. obovatus* and *B. phoenicis*			
Argentina				
Bella Vista[1]	'Hamlin' sweet orange	12	38	20
Bella Vista[2]	'Valencia' sweet orange	13	04	04
Saens Peña	Unidentified sweet orange	19	13	02
Concordia	'Valencia' sweet orange	28	61	51
Total		**89**	**143**	**95**
	Brevipalpus chilensis			
Chile				
Mostazal	*Ligustrum lucidum*	00	45	01
Santiago	*L. lucidum*	00	250	00
Total		**00**	**295**	**01**
	Brevipalpus obovatus			
Brazil				
Piracicaba	*Solanum violaefolium*	00	183	02
Total		**00**	**183**	**02**
	Brevipalpus phoenicis			
Argentina				
Bella Vista[1]	*Rhododendron* sp.	26	00	00
	'Nova' mandarin	22	00	00
Brazil				
Cordeirópolis	53 populations kept on orange fruits	1124	00	06
Piracicaba	Population kept on orange fruits	271	00	02
Costa Rica				
At the Panama border	Sweet orange	12	00	00
Paraguay				
Filadelfia	Sweet orange	28	00	00
Neuland	Sweet orange	21	00	00
Total		**1504**	**00**	**08**

[1]Estación Experimental Agropecuaria, Instituto Nacional de Tecnologia Agropecuaria; [2]Colonia Tres de Abril

with a prevalence of the former (43 versus 27%), in the 327 examined adult females by scanning electron microscopy. Examination by light microscope of 62 examined adult females showed essentially the same result. Surprisingly, in the remaining adult females, solenidion ω' was present on tarsus of leg II of one side, but absent on tarsus of leg II of the other side (Fig. 1). These mites are referred to as "asymmetric", and corresponded to about 29% of the adult females examined.

Of all the remaining 1,993 adult females examined in this study, from populations collected on 'Nova' mandarin (*Citrus reticulata* Blanco) and *Rhododendron* sp. from Bella Vista, Argentina, and on sweet orange and other hosts from other countries, only 11 were asymmetric, i.e. 0.6%. In each of those populations, a single typical *Brevipalpus* species was found (*Brevipalpus chilensis* Baker, *B. obovatus* or *B. phoenicis*).

Discussion

The examination of the samples by SEM complemented well the use of LM, given that solenidia accidentally removed during specimen preparation were detected with SEM by the visualization of its basal socket, which is much more difficult to determine with LM.

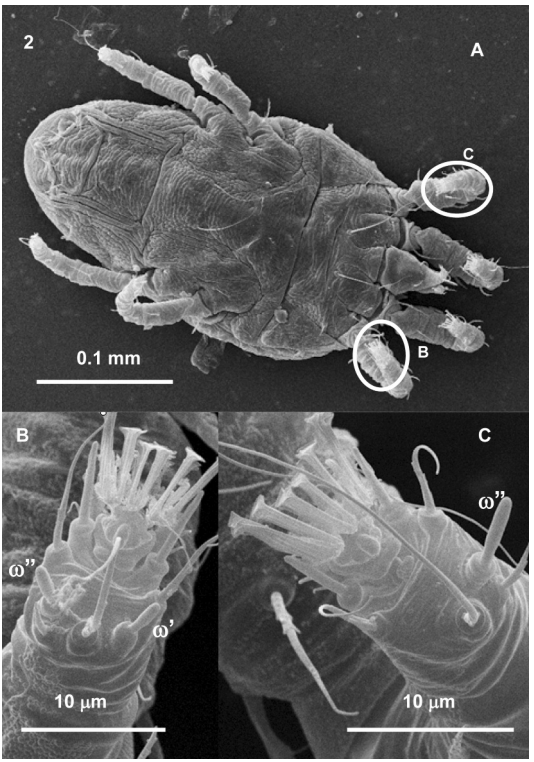

FIGURE 1. Scanning electron micrographs of *Brevipalpus* mites collected from citrus leprosis-infected Valencia sweet orange (*Citrus sinensis* [L.] Osbeck) from Bella Vista (Province of Corrientes) and Concordia (Province of Entre Rios), Argentina, revealing asymmetry in the number of solenidia on the tarsi of leg II. (A) ventral view of a mite collected at Bella Vista; details of the circled tarsi of the leg II (left and right respectively) are shown in B (two solenidia ω’ and ω”) and C (one solenidion ω”)

The observed asymmetry may correspond to “vertition”, defined as a meristic variation for a bilateral integumental organ assumed to be based on a separate genetic control for each body side (Grandjean, 1952; Leponce *et al*., 2005). Asymmetry of *Brevipalpus* was previously reported by De Leon (1967) and Welbourn *et al*. (2003). The latter authors mentioned that R. Ochoa (unpub. data) observed 23 asymmetric *Brevipalpus* specimens out of more than 6,000 specimens of *B. phoenicis* from Florida-USA, Argentina, Colombia and Costa Rica. That corresponds only to about 0.4% of the specimens, a proportion slightly lower than determined in this study for mites on plants with a single *Brevipalpus* species (0.9%).

It is remarkable that a high proportion (29%) of asymmetric adult females was found only in Argentina, on sweet orange plants where *B. obovatus* and *B. phoenicis* occurred together, and that asymmetry was rare for mites on plants hosting a single species of *Brevipalpus*. This was observed even within a relatively restricted region; in the experiment station at Bella Vista (EEABV); asymmetry was observed at high proportions on sweet orange trees, where *B. obovatus* and *B. phoenicis* occurred together, but at low proportions on other plants, onto which only *B. phoenicis* was found. This suggests that the occurrence of asymmetry depends upon the simultaneous infestation by *B. obovatus* and *B. phoenicis*, and that asymmetric mites could correspond to hybrids of those species.

Yet, the limited information available on the reproduction of *Brevipalpus* species indicates that they reproduce by thelytokous parthenogenesis, with very few males (less than 1%) in natural populations (Childers *et al*., 2003). Weeks *et al*. (2001) demonstrated that the two chromosomes of females of *B. phoenicis* correspond to a haploid condition, and that their feminization is caused by the bacterial symbiont identified as *Candidatus* Cardinium. This bacterium infects most of the tissues of *Brevipalpus* mites, including ovary and eggs (Kitajima *et al*., 2007). Eggs infected by the symbiont become females, while others become males. However, the latter are considered unable to inseminate females because their ejaculatory duct is too narrow (Pijnacker *et al*., 1981). Furthermore, recent anatomical studies showed an apparent abnormality of spermatozoa in the testes and sperm pump of male *Brevipalpus* species (Alberti & Kitajima, 2010). This information does not support the hypothesis that hybridization accounts for the observed asymmetry.

Further studies are required to verify whether the observed asymmetry is transmitted to subsequent generations, whether, despite contrary evidence, asymmetric mites could in fact correspond to hybrids of *B. phoenicis* and *B. obovatus*, and whether asymmetric mites differ from symmetric ones in their ability to transmit leprosis virus.

Acknowldegments

This work was supported by FAPESP (proc. 2008/52691-9) and CNPq (47.1705/2009-8). A.D. Tassi has a FAPESP "Iniciação Científica" scholarship. The authors express their gratitude to Roberto Trincado (SAG, Chile), W. Villalobos (Universidad de Costa Rica), C. Omoto (ESALQ/USP) and Luis G. Segagna (Universidad National de Asunción) for providing specimens of *Brevipalpus* for evaluations in this work.

References

Alberti, G. & Kitajima, E.W. (2010) Anatomy of the *Brevipalpus* mites (Tenuipalpidae, Prostigmata, Actinotrichida). In: Moraes, G.J. de, Castilho, R.C. & Flechtmann, C.H.W. (orgs) *Abstract Book of the 13th International Congress of Acarology,* Recife, pp. 8.

Childers, C.C. & Derrick, K.S. (2003) *Brevipalpus* mites as vectors of unassigned rhabdoviruses in various crops. *Experimental and Applied Acarology*, 30, 1–231.

Childers, C.C., French, J.V. & Rodrigues, J.C.V. (2003) *Brevipalpus californicus, B. obovatus, B. phoenicis,* and *B. lewisi* (Acari: Tenuipalpidae): a review of their biology, feeding injury and economic importance. *Experimental and Applied Acarology*, 30, 5–28.

De Leon, D. (1967) *Some mites of the Caribbean Area*. Allen Press Inc., Lawrence.

Frezzi, M.J. (1940) "Lepra explosiva" of orange. Studies in the pathology laboratory of Bella Vista (Corrientes). *Boletim de Frutas y Hortalizas*, 5, 1–15.

Gonzales, R.H. (1975) Revision of the *Brevipalpus phoenicis* "complex" with descriptions of new species from Chile and Thailand (Acarina, Tenuipalpidae). *Acarologia*, 17, 81–91.

Grandjean F. (1952) Sur les variations individuelles. Vertitions (écarts) et anomalies. *Comptes Rendus de l'Académie des Sciences, Series III*, 235, 640–643.

Kitajima, E.W., Chagas, C.M. & Rodrigues, J.C.V. (2003) *Brevipalpus*-transmitted plant virus and virus-like diseases: cytopathology and some recent cases. *Experimental and Applied Acarology*, 30, 135–160.

Kitajima, E.W., Groot, T.V.M., Novelli, V.M., Freitas-Astua, J., Alberti, G. & Moraes, G.J. de (2007) *In situ* observation of the *Cardinium* symbionts of *Brevipalpus* (Acari: Tenuipalpidae) by electron microscopy. *Experimental and Applied Acarology*, 42, 263–271.

Leponce, M., Noti, M-I., Bauchau, V. & Wauthy, G. (2005) 'Vertition' of integumental organs in mites revisited: a case of fluctuating asymmetry. *Comptes Rendus de l'Académie des* Sciences, *Sciences de la vie*, 324, 425–431.

Marchionatto, J.B. (1935) Argentine Republic: New studies on the "lepra explosiva" of the orange. *International, Bulletin of Plant Protection*, 9, 173–175.

Mesa, N.C., Ochoa, R., Welbourn, W.C., Evans, G.A. & Moraes, G.J. de (2009) A catalogue of the Tenuipalpidae (Acari) of the world with a key to genera. *Zootaxa*, 2098, 1–185.
Musumeci, M.R. & Rossetti, V. (1963) Transmissão de sintomas de leprose dos citros pelo ácaro *Brevipalpus phoenicis*. *Ciência e Cultura*, 15, 228.
Pijnacker, L.P., Ferwerda, M.A. & Helle, W. (1981) Cytological investigations on the female and male reproductive system of the parthenogenetic privet mite, *Brevipalpus obovatus* (Donnadieu) (Phytoptipalpidae, Acari). *Acarologia*, 22, 157–163.
Spegazzini, C. (1920) Sobre algunas enfermedades y hongos que afectan plantas de "agrios" en el Paraguay. *Annales de la Sociedad Científica Argentina*, 90, 155–188.
Vergani, A.R. (1945) *Transmision y naturaleza de la "lepra explosiva" del naranjo*, Ministerio de Agricultura de la Nacion, Instituto Sanidad Vegetal, 5, pp. 1–11.
Weeks, A., Marec, F. & Breeuwer, J.A.J. (2001) A mite species that consists entirely of haploid females. *Science*, 292, 2479–2482.
Welbourn, W.C., Ochoa, R., Kane, E.C. & Erbe, E.F. (2003) Morphological observations on *Brevipalpus phoenicis* (Acari: Tenuipalpidae) including comparisons with *B. californicus* and *B. obovatus*. *Experimental and Applied Acarology*, 30, 107–133.
Zeman, V. (1932) *Lepra explosiva del naranjo*. Universidade Nacional del Litoral. Facultad de Agronomia, Ganadeíra y Industrias Afines, Corrientes.