Diaphorina citri (Kuwayama, 1907) and *Tamarixia radiata* (Waterson, 1922) in citrus crops of Cundinamarca, Colombia

Diaphorina citri (Kuwayama, 1907) y *Tamarixia radiata* (Waterson, 1922) en cítricos en el departamento de Cundinamarca, Colombia

Everth Emilio Ebratt-Ravelo[1, 6], Leidy Tatiana Rubio-González[2], Valmir Antonio Costa[3], Ángela Patricia Castro-Ávila[4], Edna Milena Zambrano-Gómez[5], and Jorge Evelio Ángel-Díaz[1]

ABSTRACT

The presence of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) and *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) was recorded in citrus crops of Cundinamarca, Colombia. This work is to know the geographic distribution of *D. citri* and an initial record of the parasitoid *T. radiata* in citrus producing areas of this department.

Key words: biological control, Psyllidae, Eulophidae, citrus, *Murraya* sp.

RESUMEN

La presencia de *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) y *Tamarixia radiata* (Waterson) (Hymenoptera: Eulophidae) se registró en cultivos de cítricos en el departamento de Cundinamarca, Colombia. Igualmente se da a conocer la distribución geográfica de *D. citri* y se realiza un primer reporte del parasitoide *T. radiata* en regiones productoras de cítricos en este mismo departamento.

Palabras clave: control biológico, Psyllidae, Eulophidae, cítricos, *Murraya* sp.

Introduction

Diaphorina citri Kuwayama was reported in Colombia associated with citrus seedlings in various nurseries in the departments of Valle del Cauca and Tolima in 2007 by the Colombian Agricultural Institute (ICA). Afterwards in 2008 it was found in the town of Girardot in Cundinamarca by the same entity. In Colombia, there are approximately 60,000 ha planted in citrus of which 10,633 are in Cundinamarca (Plan Nacional Hortifrutícola (MADR, 2006). According to Halbert and Núñez (2004), *D. citri* has 59 species of the family Rutaceae as hosts, especially of the genera *Citrus* sp. and *Murraya* sp. The importance of *D. citri* is its ability to transmit the disease known as Huanglongbing or HLB (Da Graca, 1991; Van der Merwe and Andersen, 1937; Broadbent *et al*., 1977; Aubert, 1987; Liu and Tsai, 2000; Tsai and Liu, 2000; Tsai *et al*., 2002), whose etiologic agent are the bacteria *Candidatus* Liberibacter asiaticus and *Candidatus* Liberibacter americanus in America (Halbert and Núñez, 2004).

This work aimed to acquire knowledge of the life cycle and the geographic distribution of Asian psyllid *D. citri* and reported the parasitoide *Tamarixia radiata* (Waterston) in citrus-producing regions in the department of Cundinamarca in Colombia.

Materials and methods

The monitoring began in November 2008 and ended in November 2010 and included the following regions: Gualiva, Alto Magdalena, Centro Magdalena, Bajo Magdalena, Rionegro, Sumapaz, Medina, Tequendama, and the east in the department of Cundinamarca; a classification method based on zones proposed by L.R. Holdridge (1967, 1982) and the IGAC (1997) were implemented for this purpose. Seven routes were established for the collection of samples, corresponding to the most important departmental roads: Fusagasugá-Bogota-Girardot, Bogota-Girardot-La Mesa, Bogota-San Francisco-Villeta-Guaduas, Bogota-Facatativá-Sasaima-Villeta, Bogota Zipaquirá-Pacho-La Palma, Bogota-Choachi-Caqueza-Bogota and Bogota-Viani-San Juan de Rioseco.

To determine the presence of *D. citri* and its natural enemies in crops, some samples were taken from valencia orange

Received for publication: 8 February, 2011. Accepted for publication: 2 November, 2011.

1 Laboratorio Nacional de Diagnóstico Fitosanitario, Tibaitatá Research Center, Instituto Colombiano Agropecuario (ICA). Mosquera (Colombia).
2 Universidad Francisco de Paula Santander; Laboratorio Nacional de Diagnóstico Fitosanitario, Tibaitatá Research Center, Instituto Colombiano Agropecuario (ICA). Mosquera (Colombia).
3 Centro Experimental do Instituto Biológico Rodovia Heitor Penteado. Campinas, SP (Brasil).
4 Faculty of Agronomy, Universidad Nacional de Colombia. Bogota (Colombia).
5 Fruit Project, Laboratorio Nacional de Diagnóstico Fitosanitario, Tibaitatá Research Center, Instituto Colombiano Agropecuario (ICA). Mosquera (Colombia).
6 Corresponding author. everth.ebratt.ravelo@gmail.com; everth.ebratt@ica.gov.co

(*Citrus sinensis* Osbeck), mineola tangelo (*Citrus reticulata* x *C. grandis*), tangerine (*Citrus reticulata* Blanco), tahiti lime (*Citrus aurantifolia* Swingle), myrtle plantas (*Murraya paniculata*), and swinglia (*Swinglea glutinosa*), the last, used as living fences in citrus producing farms, cattle ranchs and residential condominiums.

D. citri adults were captured, the host plant was first surrounded with entomological jama, moving up and down, smoothly touching the plant leaves; the content was completely deposited in a sealable plastic bag, then it was observed and verified to assure the presence of adults inside and then were passed to vials with 70% of ethanol using a 00 caliber moistened brush. To capture immature was necessary a 20x loupe to identify infested shoots with either eggs or mass colonies of nymphs, were introduced in paper bags and then into sealable plastic bags. In each site ten plants were sampled for a total of 770 plants during the whole study. The samples were labeled with the basic data of the capture (town, village, farm, plant material, harvest date, geographic location) and to send to the National Laboratory of Plant Pathology Diagnostic– ICA Tibaitatá, located in Mosquera (Cundinamarca, Colombia, 4°41'46,57" N, 74°12'13" W and 2,544 m a.s.l.), where they were maintained at an average temperature of 24°C until the adult emergence of *D. citri* or its parasitoides from *D. citri* nymphs.

With the insect material of *D. citri* obtained in confinement, 10 seedlings of mandarin were infested, with a pair of *D. citri* per plant and kept in cages of 3 x 3 x 3 m with a swiss veil on top, at an average temperature of 22.27 ± 2.35°C. From the appearance of the first mass of eggs in each plant, the adults were removed; two daily readings were done (08:00 h and 16:00 h) according to the methodology proposed by Liu and Tsai (2000), Tsai and Liu, (2000), Tsai *et al.* (2002), Gómez and Postali (2009), for a period of 60 d. This also allowed determining the developmental stages of *D. citri*, their morphological, morphometrica, and the time between the different instants of development.

In order to identify and characterize the states of development of *D. citri* and to obtain parasitoids emerged from pupae, the proposed methodology was the one implemented by Gómez y Postali (2009). The entomological material for adults of *D. citri* and *T. radiata,* were confirmed to species by Dr. Daniel Burckhardt (Entomologie Naturhistorisches Museum Basel Switzerland) and Dr. Valmir A. Costa (Centro Experimental do Instituto Biológico Campinas, Brasil), respectively.

Results and discussion

Geographical distribution and hosts

The presence of *D. citri* Kuwayama was detected in 16 of 20 local town producers of citrus crops established in orange, tangelo, tangerine, lime Tahiti, swinglia myrtle plants in altitude and temperature ranges average between 250 to 1,900 m and 35°C to 18°C respectively, with bimodal rainfall (650 to 3000 mm per year) in the inter-Andean region of the Magdalena River and unimodal in the eastern region (700 to 3,000 mm $year^{-1}$).

In the department of Cundinamarca, *D. citri* is present in 60% of the citrus-producing regions and involved three life zones for tropical dry forest (bs-T), premontane dry forest (bs-PM) and premontane wet forest (bh-PM). It is not present in the region of Sasaima which is a very humid forest (bmh-PM), eventhough this zone is characterized for having the presence of host plants with the exception of myrtle, however, it is important to note that the conditions of high rainfall and low temperature, which occurred during this study could be generated adverse environmental conditions to *D. citri*, in contrast to those reported in other regions. Additionally, it is not present in the municipalities of Fómeque, Choachí, and the premontane wet forest (bh-PM), at the department eastern slope, possibly due to the temperature which is below 20°C and the low or no presence of host plants (Tab. 1).

From 770 plants sampled, 525 were infested with *D. citri*. The largest occurrence corresponded to the myrtle (*M. paniculata*) with 100% findings (190/190 plants), followed by mandarin (*C. reticulata*) with 85,71% findings (120/140 plants), orange (*C. sinensis*) with 60% findings (120/200 plants), tangelo (*C. reticulata* x *C. grandis*) with 55,55% findings (50/90 plants), Tahiti lime (*C. aurantifolia*) with 50% findings (35/70 plants), and swinglia (*S. glutinosa*) with 50% findings (40/80 plants), confirming the results obtained by Liu and Tsai, (2000); Tsai and Liu, (2000), and Tsai *et al*. (2002); however, but under field conditions, the preference may be favored by the presence of hosts, the range of feeding sites and oviposition sites for the psyllid, by way of a greater number of shoots; above as a result of specific conditions like water stress, rainfall, and temperature en each zone involved. These conditions make allow to understand how difficult it is to provide regional management of *D. citri*, if the presence, distribution and host plant preference within the management plans of their populations is not taken into account.

TABLE 1. Citrus regions and life zones in the presence of *D. citri* and *T. radiate* in the department of Cundinamarca.

Province	Municipality	Crops	Presence of species		Geographical location					
			Diaphorina citri	Tamarixia radiata	N	W	Altitude (m)	Average (°C)	Annual rainfall (mm)	Life zone
Alto Magdalena	Girardot	Naranja, Tangelo, mandarina, mirto, swingliae	+	-	04°17’55,26’’	074°48’27,45’’	281	28	1,167	Bosque seco tropical
	Tocaima	Swingliae, Naranja y mirtos	+	-	04°27’35,14’’	074°38’10,36’’	385	28	1,051	Bosque seco tropical
		Naranja, Tangelo, mandarina, mirto	+	+	04°29’35,7’’	074°38’44,7’’	435	28	1,050	Bosque seco tropical
		Swingliae, Naranja y mirtos	+	+	04°27’28,87’’	074°37’8,72’’	400	28	1,050	Bosque seco tropical
		Naranja, Mandarina y mirto	+	+	04°25’2,6’’	074°42’34,99’’	365	28	1,050	Bosque seco tropical
		Mirto, naranja, limón, swingliae	+	-	04°26’54,56’’	074°34’41,2’’	441	28	1,050	Bosque seco tropical
		Mirto, naranja, limón	+	-	04°26’40,78’’	074°34’4,94’’	460	28	1,050	Bosque seco tropical
	Agua de Dios	Mirto, naranja, limón, mandarina	+	-	04°22’32,49’’	074°40’12,13’’	370	27	1,000	Bosque seco tropical
		Naranja, tangelo, mirto	+	-	04°20’16,1’’	074°42’14,8’’	344	27	1,000	Bosque seco tropical
		Lima Acida Tahiti, mirto	+	-	04°23’41,2’’	074°39’10,7’’	356	27	1,000	Bosque seco tropical
	Jerusalen	Naranja, tangelo, mirto	+	-	04°33’47,62’’	074°41’43,80’’	306	27	996	Bosque seco premontano
	Beltran	Lima Acida Tahiti, mirto	+	-	04°47’37,67’’	074°44’14,06’’	277	27	922	Bosque seco premontano
		Lima Acida Tahiti, mirto	+	-	04°46’21,86’’	074°48’20,92’’	249	27	922	Bosque seco premontano
Sumapaz	Fusagasuga	Naranja, tangelo, mandarina	+	-	04°20’20,64’’	074°21’48,72’’	1,760	20	1,250	Bosque húmedo premontano
	Silvania	Naranja, mandarina	+	-	04°24’15,47’’	074°23’12,10’’	1,484	20	1,760	Bosque húmedo premontano
	Pandi	Naranja, limon	+	-	04°11’59,78’’	074°39’00,76’’	317	25	1,250	Bosque seco tropical
	Arbelaez	Naranja Tangelo	+	-	04°16’00,31’’	074°24’59,77’’	1,34	20	1,380	Bosque húmedo premontano
Tequendama	Anapoima	Naranja, Tangelo, mandarina	+	-	04°33’02,50’’	074°32’08,75’’	675	25	1,300	Bosque húmedo premontano
	La Mesa	Naranja, Tangelo, mandarina, mirto	+	-	04°37’50,86’’	074°27’43,56’’	1,273	25	2,500	Bosque húmedo tropical
	Tena	Naranja Tangelo, mandarina	+	-	04°39’01,87’’	074°23’39,49’’	1,259	23	3,000	Bosque muy húmedo premontano
	Apulo	Naranja	+	-	04°31’04,55’’	074°35’50,79’’	447	28	1,600	Bosque seco tropical
	Viotá	Naranja, mirto, limón swingliae, mandarina	+	-	04°26’00,17’’	074°31’00,62’’	567	25	1,600	Bosque seco tropical
		Mirto (Murraya Paniculata), mandarina, swingliae	+	-	04°26’49,67’’	074°35’6,72’’	386	25	1,600	Bosque seco tropical
		Naranja, mirto, mandarina, swingliae	+	-	04°26’49,67’’	074°34’16,68’’	437	25	1,600	Bosque seco tropical
Rio Negro	Pacho	Mirto (Murraya paniculata)	+	-	05°08’00,00’’	074°09’59,57’’	1,854	19	1,556	Bosque húmedo premontano
		Mirto (Murraya paniculata)	+	-	05°8’22,02’’	074°9’30,74’’	1,653	19	1,556	Bosque húmedo premontano
Madalena Centro	San J. Rio Seco	Naranja	+	-	04°50’54,74’’	074°37’16,12’’	1,269	21	1,230	Bosque húmedo premontano
Gualiva	La Vega	Limon swingliae	+	-	04°58’29,48’’	74°19’40,62’’	1,293	22	2,000	Bosque húmedo premontano
	Sasaima	Mandarina, naranja	-	-	04°57’37,51’’	74°25’27,01’’	1,191	24	2,400	Bosque muy húmedo premontano
	San Francisco	Mandarina, naranja	-	-	04°58’29,48’’	074°17’21,62’’	1,500	20	1,493	Bosque húmedo premontano
Oriente	Choachi	Naranja	-	-	04°32’00’’	73°56’00’’	1,600	18	922	Bosque húmedo premontano

Life cycle of *D. citri*

The insect showed the following life cycle stages: it was first in its egg, then five nymph stages and a final adult stage which took place over a period of 26.88±8.23 d to 22.27±2.35°C (Tmax= 27.37°C and Tmin= 18.04°C) (Fig. 1, Fig. 2), with are differential characteristics in morphology and morphometry, according to Gómez and Postali (2009), that enable the characterization process of each of these stages of development (Tab. 2, Tab. 3), as observed by Liu and Tsai (2000), Tsai and Liu (2000), and Tsai *et al.* (2002), who determined the life cycle of *D. citri* is full filled in 28.6 ± 4.5 d at optimum temperatures of 25 and 28°C, and a significant reduction in oviposition, at temperatures outside these ranges.

Worldwide, *Tamarixia radiata* (Waterston) is among the most potentially beneficial organisms for biological control of *D. citri*. *T. radiata* (Waterston) is a microhimenophtero of 1.43 mm (n=4; Ds= 0.3513) and 1.20 mm (n=4; Ds= 0.2008) in length in females and males respectively, which acts as a ectoparasitoide idiobionte in nymph III, nymph IV and nymph V of *D. citri* (Gómez and Postali, 2009; Chien *et al.*, 2001). According to Chien *et al.* (2001), the female lays egg on the ventral part of the nymph III, between the thorax and the abdomen, the newly hatched larva sucks hemolymph from the outer zone which is attached to host integument, then the parasitoid is dragged to the ventral region of the chest and still feeding. When *T. radiata* fulfills all its development and eats the whole hemolymph of its host, the latter gets mummified and inside the mature parasitoid larva transforms into a prepupa and finnaly into a pupa. According to Gómez and Postali (2009), the life cycle of *T. radiata* from egg to adult emergence is accomplished in 12 d at 25°C or in 8 d at 30°C, and has longevity of 60 d at 20°C (Quilici and Fauvergue, 1990; Fauvergue and Quilici, 1991).

The nymphs of *D. citri* parasitized by *T. radiata* (Fig. 3), were obtained from vegetable material of mandarin and orange from Tocaima (04°29'35,7" N, 74°38'44,7" W y 435 msnm; 04°27'28,87" N, 74°37'8,72" W y 400 msnm; 04°25'2,6" N, 74°42'34,99" W y 365 msnm), located in the zone of tropical

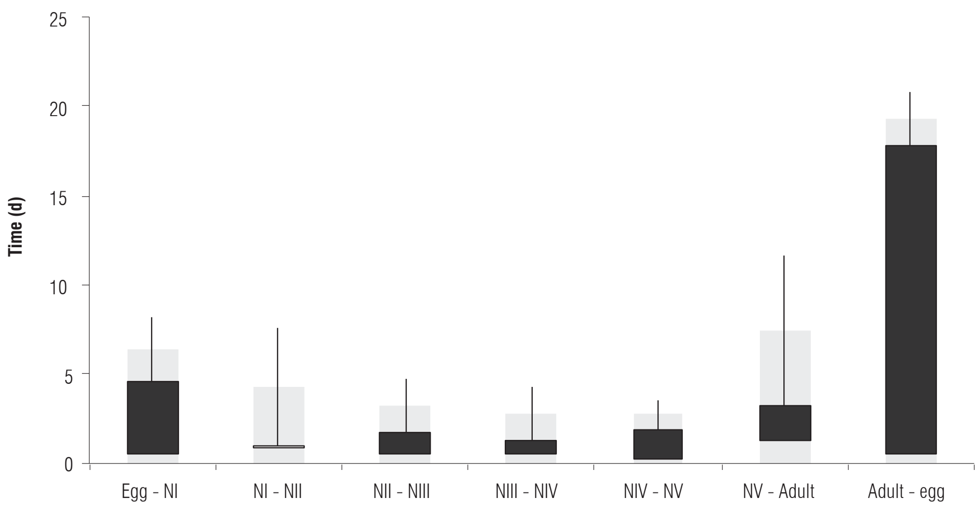

FIGURE 1. Life cycle of *D. citri* for six instars and stages of development, under controlled laboratory conditions (22,27±2,35°C).

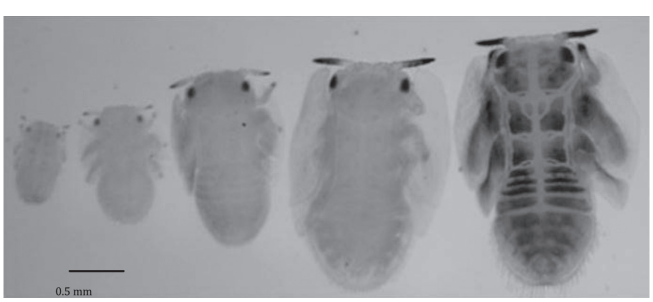

FIGURE 2. Nymphal Instars of *D. citri* (from left to right) Nymph I to V. Source: E. Ebratt.

TABLE 2. Stages of development of *D. citri* and elapsed time in days.

Stages	n	Mean	-95.00%	95.00%	SD	SE
Egg - NI	10	6.38	4,611	8.139	1.109	0.554
NI - NII	10	4.25	0.970	7.530	2.062	1.031
NII - NIII	10	3.25	1.727	4.773	0.957	0.479
NIII - NIV	10	2.75	1.227	4.273	0.957	0.479
NIV - NV	10	2.75	1.954	3.546	0.500	0.250
NV - Adult	10	7.50	3.290	11.710	2.646	1.323
Adult - egg	10	19.25	17.727	20.773	0.957	0.479

TABLE 3. Morphometry characteristics of stages of development of *D. citri.*

Stages	Valid N	Mean mm	Confidence	Confidence	Minimum	Maximum	Variance	SD	SE
Males									
Total length	29	2.809310	2,744862	2.873758	2.270000	3.050000	0.028707	0.169430	0.031462
Front width	29	0.140000	0.135020	0.144980	0.120000	0.170000	0.000171	0.013093	0.002431
head	29	0.286207	0.277034	0.295380	0.240000	0.350000	0.000582	0.024115	0.004478
antenna length	29	0.403448	0.388360	0.418536	0.310000	0.470000	0.001573	0.039666	0.007366
wing length	29	2.177931	2.147477	2.208385	2.030000	2.350000	0.006410	0.080062	0.014867
Females									
Total length	33	2.973939	2.933240	3.014639	2.780000	3.250000	0.013175	0.114781	0.019981
Front width	33	0.132727	0.128639	0.136816	0.100000	0.160000	0.000133	0.011531	0.002007
head	33	0.293636	0.284096	0.303176	0.230000	0.340000	0.000724	0.026905	0.004684
antenna length	33	0.414545	0.395802	0.433289	0.300000	0.520000	0.002794	0.052861	0.009202
wing length	33	2.286364	2.256436	2.316292	2.120000	2.430000	0.007124	0.084403	0.014693
Nymph I									
Total length	10	0.576000	0.502489	0.649511	0.450000	0.700000	0.010560	0.102762	0.032496
right antenna length	10	0.094000	0.076413	0.111587	0.070000	0.140000	0.000604	0.024585	0.007775
left antenna length	10	0.092000	0.076255	0.107745	0.070000	0.130000	0.000484	0.022010	0.006960
Head width	10	0.238000	0.205166	0.270834	0.190000	0.300000	0.002107	0.045898	0.014514
Fronto antennal width	10	0.347000	0.301625	0.392375	0.270000	0.430000	0.004023	0.063430	0.020058
Nymph II									
Total length	10	0.717000	0.679745	0.754255	0.650000	0.790000	0.002712	0.052079	0.016469
right antenna length	10	0.124000	0.108111	0.139889	0.080000	0.150000	0.000493	0.022211	0.007024
left antenna length	10	0.118000	0.101549	0.134451	0.080000	0.150000	0.000529	0.022998	0.007272
Head width	10	0.301000	0.258955	0.343045	0.250000	0.460000	0.003454	0.058775	0.018586
Fronto antennal width	10	0.393000	0.356987	0.429013	0.300000	0.460000	0.002534	0.050343	0.015920
Nymph III									
Total length	10	1.036000	0.980261	1.091739	0.900000	1.110000	0.006071	0.077917	0.024640
right antenna length	10	0.174000	0.157757	0.190243	0.140000	0.220000	0.000516	0.022706	0.007180
left antenna length	10	0.169000	0.151988	0.186012	0.130000	0.210000	0.000566	0.023781	0.007520
Head width	10	0.399000	0.385745	0.412255	0.380000	0.430000	0.000343	0.018529	0.005859
Fronto antennal width	10	0.600000	0.572192	0.627808	0.530000	0.670000	0.001511	0.038873	0.012293
Nymph IV									
Total length	10	1.444000	1.365405	1.522595	1.270000	1.560000	0.012071	0.109869	0.034744
right antenna length	10	0.270000	0.247128	0.292872	0.230000	0.320000	0.001022	0.031972	0.010111
left antenna length	10	0.254000	0.230346	0.277654	0.200000	0.310000	0.001093	0.033066	0.010456
Head width	10	0.530000	0.514183	0.545817	0.500000	0.570000	0.000489	0.022111	0.006992
Fronto antennal width	10	0.804000	0.766722	0.841278	0.740000	0.880000	0.002716	0.052111	0.016479
Nymph V									
Total length	10	1.596000	1.541395	1.650605	1.510000	1.770000	0.005827	0.076333	0.024138
right antenna length	10	0.269000	0.251988	0.286012	0.210000	0.300000	0.000566	0.023781	0.007520
left antenna length	10	0.258000	0.235682	0.280318	0.200000	0.310000	0.000973	0.031198	0.009866
Head width	10	0.531000	0.509819	0.552181	0.480000	0.570000	0.000877	0.029609	0.009363
Fronto antennal width	10	0.851000	0.806205	0.895795	0.710000	0.910000	0.003921	0.062619	0.019802

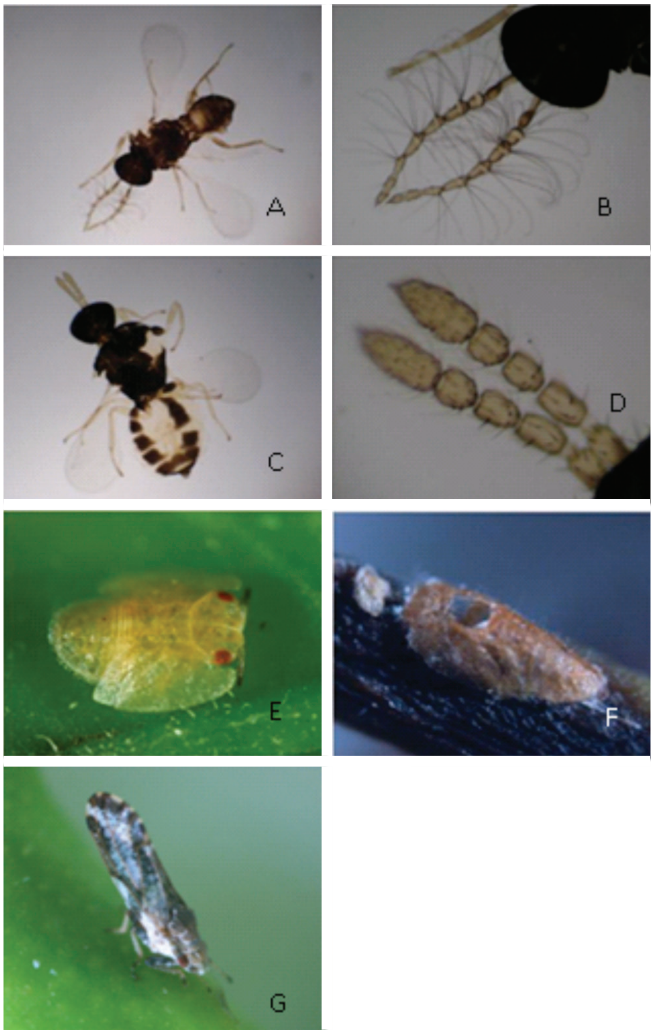

FIGURE 3. A. *Tamarixia radiata* male adult; B. Antennas *T. radiata* male; C. *T. radiata* female adult; D. Antennas *T. radiata* female; E. *D. citri* nymph V; F. *D. citri* nymph V parazited-hatched for *T. radiata*; G. *D. citri* adult. Source: Tatiana Rubio and Everth Ebratt.

dry forest (bs-T), characterized by temperatures of 28°C and 1,050 mm mean annual precipitation, and a 15% of natural level of parasitoidism. These thermal-hygrometric conditions correspond to the optimal considered for *T. radiata* reported by Gómez and Postali (2009) in the state of Sao Paulo (Brazil), suggesting the dependence of the parasitoid to these environmental conditions for dispersion, colonization, and establishment in areas with *D. citri* presence.

Also, Aubert (1987) reported these conditions and he found levels of 80% parasitoidism in *D. citri* by inoculation of parasitoid of citrus crops in India, the Arabian Peninsula, Philippines, Thailand and Indonesia from entomological material that grown massively. According to Chien *et al.* (1991a, 1991b, 1995), the mass breeding of *T. radiate* permits inundative introductions of crops infested by *D.citri*, with more effective parasitoidism levels to those exercised by the natural parasitoidism.

Conclusions

D. citri is present in 60% of the citrus-producing areas of the Andean region in the department of Cundinamarca and has become the main phytosanitary problem of the citrus production in the department, being the main transmitter of the disease known as HLB.

The presence of *T. radiata* was reported as a natural enemy of *D. citri* colonizing citrus producing regions located in andean areas of tropical dry forest of the department of Cundinamarca and its presence is an alternative biological control management for the psyllid vector of HLB to the citriculture in Colombia.

Acknowledgments

To Instituto Colombiano Agropecuario – ICA, Cundinamarca seccional, local offices Girardot, Pacho, Caqueza, Fusagasuga, La Meza, Dr. Jorge Evelio Angel Díaz National Laboratory of Plant Pathology Diagnostic, Dr. Daniel Burckhardt for diagnostic confirmation the asian psyllid, and Dr. Valmir A. Costa (Centro Experimental do Instituto Biológico, Campinas Brasil) to confirm the *T. radiata* diagnostic.

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