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Source: Journal of Economic Entomology, 105(2):329-337. 2012.
Published By: Entomological Society of America
DOI: http://dx.doi.org/10.1603/EC11254
URL: http://www.bioone.org/doi/full/10.1603/EC11254

Weedy Hosts and Prevalence of Potential Leafhopper Vectors (Hemiptera: Cicadellidae) of a Phytoplasma (16SrIX group) Associated With Huanglongbing Symptoms in Citrus Groves

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J. Econ. Entomol. 105(2): 329–337 (2012); DOI: http://dx.doi.org/10.1603/EC11254

ABSTRACT Huanglongbing (HLB) is a severe citrus (*Citrus* spp.) disease associated with the bacteria genus *Candidatus* Liberibacter, detected in Brazil in 2004. Another bacterium was found in association with HLB symptoms and characterized as a phytoplasma belonging to the 16SrIX group. The objectives of this study were to identify potential leafhopper vectors of the HLB-associated phytoplasma and their host plants. Leafhoppers were sampled every other week for 12 mo with sticky yellow cards placed at two heights (0.3 and 1.5 m) in the citrus tree canopy and by using a sweep net in the ground vegetation of two sweet orange, *Citrus sinensis* (L.) Osbeck, groves infected by the HLB-phytoplasma in São Paulo state. Faunistic analyses indicated one Agalliinae (*Agallia albidula* Uhler) and three Deltocephalinae [*Balclutha hebe* (Kirkaldy), *Planicephalus flavicosta* (Stål), and *Scaphytopius* (*Convelinus*) *marginelineatus* (Stål)] species, as the most abundant and frequent leafhoppers (Hemiptera: Cicadellidae). Visual observations indicated an association of leafhopper species with some weeds and the influence of weed species composition on leafhopper abundance in low-lying vegetation. *S. marginelineatus* and *P. flavicosta* were more frequent on *Sida rhombifolia* L. and *Althernantera tenella* Colla, respectively, whereas *A. albidula* was observed more often on *Conyza bonariensis* (L.) Cronq. and *B. hebe* only occurred on grasses. DNA samples of field-collected *S. marginelineatus* were positive by polymerase chain reaction and sequencing tests for the presence of the HLB-phytoplasma group, indicating it as a potential vector. The association of leafhoppers with their hosts may be used in deciding which management strategies to adopt against weeds and diseases in citrus orchards.

KEY WORDS weed, plant disease, phloem-limited bacteria, sap-sucking insect

Many pests and diseases can inflict significant damage to the Brazilian citriculture, including Huanglongbing (HLB), one of the most destructive diseases of citrus (*Citrus* spp.). Until recently, this disease was associated only with the bacteria *Candidatus* Liberibacter asiaticus and *Ca.* L. americanus, both transmitted by the Asian citrus psyllid, *Diaphorina citri* Kuwayama, and with *Ca.* L. africanus, transmitted by the psyllid *Trioza erytreae* (Del Guercio) (Bové 2006, Yamamoto et al. 2006, Gottwald 2010). However, in 2008 a new agent was identified in orange trees with HLB symptoms that were negative for the presence of *Ca.* Liberibacter spp. It was a phytoplasma with a 99% similarity with Pigeon pea witches' broom phytoplasma of the 16Sr IX group (Teixeira et al. 2008). The phytoplasmas are prokaryotes with no cell wall and size variation between 200 and 800 nm, important pathogenic agents transmitted by insects (mostly leafhoppers but also planthoppers and psyllids), causing ≈700 diseases in plants, many of which are lethal (Weintraub and Beanland 2006, Bertaccini 2007).

Until now, Hemiptera is the only order identified as having phytoplasma vectors and presenting several characteristics that make its members efficient vectors of these plant pathogens. Adults and nymphs generally have similar feeding habits; consequently, both stages can acquire and transmit phytoplasmas. Some hemipteran groups feed specifically and selectively in the phloem of their host plants, allowing acquisition and inoculation of phytoplasmas that inhabit this plant tissue. In addition, the phytoplasmas have a persistent and propagative relationship with insects, similar to other symbiotic prokaryotes found in hemipterans (Weintraub and Beanland 2006, Weintraub 2007).

There are more studies on the identification and molecular characterization of phytoplasmas than on the determination of vectors for this pathogen group (Weintraub and Beanland 2006). Because many phytoplasma vectors are still unknown, as it is the case of the phytoplasma associated with HLB, the surveying of leafhopper species present in commercial crops is

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fundamental for knowing the potential of some species to transmit these pathogens. Ongoing studies suggest that the diversity of leafhoppers in Brazil guarantees many options of potential vector species (Yamamoto and Gravena 2000, Lopes and Oliveira 2004, Giustolin et al. 2009, Miranda et al. 2009b, Ringenberg et al. 2010).

Other important information, such as alternative host plants for the leafhoppers, are necessary for the implementation of management strategies. However, these same plants can be hosts to phytoplasmas because various taxonomic plant groups are infected by these pathogens (Montano et al. 2007, Eckstein 2010).

In the search for information on potential vectors of the HLB-associated phytoplasma (phytoplasma-HLB) and their host plants, the aim of the current study was to identify the leafhopper species present in citrus groves affected by this pathogen; associate the presence of weeds with the populations of these insects, as well as the importance of these plants in the area; and to evaluate the occurrence of leafhoppers species carrying the pathogen.

Materials and Methods

Survey of Leafhoppers. The survey of leafhoppers was made in the Nova Granada municipality, northern São Paulo state, Brazil. This area was selected by the presence of phytoplasma infected plants of the 16SrIX group, associated with HLB symptoms and confirmed by polymerase chain reaction (PCR) and sequencing. Two areas of sweet oranges were sampled [*Citrus sinensis* (L.) Osbeck], one area composed of 'Valência' plants grafted onto 14-yr-old rangpur lime rootstock (*Citrus limonia* Osbeck) (plot A, 20°31′48″S, 49°12′36″W) and the other area with 'Pêra' plants grafted onto 8-yr-old rangpur lime rootstock (plot B, 20°33′27″S, 49°18′02″W). Plot A (1.5 ha) is triangular and borders an area of semideciduous seasonal forest on one side and with orange orchards bordering the other sides. Plot B (1 ha) is also triangular, with a rubber tree plantation on one side and orange orchards on the other sides. The tree spacing in both plots was 6 by 7 m.

The survey methodology of potential vector species was developed for collecting leafhoppers (Hemiptera: Cicadellidae), which constitute the main group of phytoplasma vectors (Weintraub and Beanland 2006). One method for sampling leafhoppers was the use of a sweep net in the two study plots every 2 wk between April 2008 and May 2009. Collections were made in the morning between 0900 and 1100 hours. Twenty points at random in intertree rows were swept in the plots and on low-lying vegetation, with each point composed of 50 sweeps with the net. The insects were kept in a freezer (−20°C) for ≈24 h until the morphotypes were separated. Some of the insects collected were deposited in a reference collection and the rest were used to extract DNA and for the PCR testing. The objective of the PCR test was to detect the phytoplasma-HLB in the field-collected leafhoppers.

Sticky yellow cards (both sides, measuring 8.5 by 11 cm) were used in the two study areas to evaluate leafhopper activity. These traps were set out at 20 points within each plot, with each point consisting of one citrus plant with two traps fastened to the tree side-branches at heights of 0.3 and 1.5 m. These two heights were chosen to investigate leafhopper activity in the canopy of the orange trees or moving within the orchard (1.5 m in height) and also on the ground vegetation (0.3 m). Captured leafhoppers were removed from the sticky cards by using a solvent (kerosene) to dissolve the glue and were mounted on insect pins.

Insect identification was made under a stereoscopic microscope, comparing the morphological characteristics with those of leafhopper specimens already identified from a reference collection at the Entomology Museum of the Department of Entomology and Acarology, ESALQ/University of São Paulo (USP). Several specimens of each morphospecies collected in the survey were sent to Dr. Keti Zanol (Department of Zoology, Federal University of Paraná, Paraná, Brazil), a specialist in Deltocephalinae, for taxonomic identification. Voucher specimens of each species were deposited at the Entomology Museum of ESALQ/USP. Based on the number of leafhoppers collected from sticky traps in each plot for the May 2008–May 2009 period, a faunistic analysis was made to determine those species that had the highest indices of constancy, frequency, abundance, and dominance (Silveira Neto et al. 1995).

Collection and Identification of Weeds in the Plots. A botanical survey of weeds was made in the study plots. A 1- by 1-m (1-m^2) square was thrown five times randomly between the orange tree rows and five times beneath the tree canopies, for a total sample area of 10 m^2 in each plot. In the area delimited by the square, the number of plants was counted and each identified using taxonomic keys (Kissmann and Groth 2000). From the results it was possible to calculate the frequency, density, abundance, relative frequency, relative density, relative abundance, and importance value index (IVI) (Mueller-Dombois and Ellenberg 1974). The IVI value is the sum of the other indices and was used to rank the importance of each plant species present in the area.

Association of Leafhoppers With Weed Hosts. Those leafhoppers that were frequent and abundant were visually associated with invasive plants in the sweet orange orchard. Invasive plants with a high IVI, and identified as important in the areas, were observed for ≈30 s, registering the leafhopper species and number of individuals on each plant. Five examples of each plant species were observed. Some leafhopper specimens observed in the visual inspections were collected to confirm the species identification. The association between the species of invasive plants and leafhoppers was evaluated by a chi-square test using R 2.12.1 software (R Development Core Team 2011).

Detection of Phytoplasma Associated With Citrus Huanglongbing in Insects. The insects collected with the sweep net were separated in groups of 10 indi-

Table 1. Hopper species collected with a sweep net on low-lying vegetation between tree rows in areas of sweet oranges, 14 (plot A) and 8 (plot B) yr old, Nova Granada–São Paulo

Family, subfamily	Plot A		Plot B	
	Quantity[a]	%	Quantity[a]	%
Cicadellidae, Agalliinae				
Agallia albidula Uhler	110	5.6	23	4.8
Cicadellidae, Deltocephalinae				
Balclutha hebe (Kirkaldy)	793	40.6	112	23.3
Chlorotettix minimus Baker	82	4.2	25	5.2
Planicephalus flavicosta (Stål)	436	22.3	135	28.1
Scaphytopius marginelineatus (Stål)	425	21.8	126	26.3
Cicadellidae, Typhlocibinae				
Protalebrela brasiliensis Baker	29	1.5	22	4.6
Cicadellidae, Gyponinae				
Curtara samera DeLong & Freytag	31	1.6	15	3.1
Delphacidae				
Tagosodes orizicola (Muir)	45	2.3	22	4.6
Total	1,951	100.0	480	100.0

[a] Sum of 22 samples, each composed of 50 sweeps at 20 points at random in each area, between 20 April 2008 and 20 May 2009.

viduals and tested by PCR for the presence of the phytoplasma-HLB (group 16SrIX). DNA was extracted using Murray and Thompson (1980) protocol. The total DNA of each sample was used in the PCR test. Because control insects (positive and negative) were not available for the groups tested, DNA from healthy orange plants kept exclusively in nurseries covered by netting was used as a negative control, and the DNA from plants infected with citrus phytoplasma from the 16SrIX group, whose identity had been previously confirmed by genetic sequencing (Teixeira et al. 2008), was used as a positive control.

All the samples were tested for the pathogen by using nested PCR, with the pairs of primers P1 and P7 (Smart et al. 1996) in the first reaction and D7r2 and D7f2 (Teixeira et al. 2008) in the second reaction. The reaction conditions were the same as those followed by Teixeira et al. (2008). The positive samples were sent for sequencing to confirm the presence of the pathogen. The PCR products were purified in a column (Wizard SV Gel kit and PCR Clean-Up System, catalog A9281, Promega, Madison, WI) and sequenced directly. In some cases, the purified PCR products were submitted to cloning in vector plasmid pGEM T Easy (pGEM T Easy Vector System, catalog A1360, Promega) and later in *Escherichia coli* DH5-α. The cloned DNA was sequenced in the laboratory of Faculdade de Ciências Agrárias Veterinárias de Jaboticabal-Universidade Estadual Paulista.

Results and Discussion

Approximately 90% of the specimens collected by sweep net in low-lying vegetation between tree rows belonged to the Deltocephalinae subfamily (Hemiptera: Cicadellidae), with *Balclutha hebe* (Kirkaldy) being the most common species, followed by *Planicephalus flavicosta* (Stål) and *Scaphytopius* (*Convelinus*) *marginelineatus* (Stål) (Table 1). There were also a significant number of specimens of *Agallia albidula* Uhler in the captures. Cicadellids belonging to the subfamilies Gyponinae and Typhlocibinae also were collected but in lower numbers. Specimens of *Tagosodes orizicola* (Muir) (Hemiptera: Delphacidae) were the only representatives of a different family of Cicadellidae (belonging to the Delphacidae).

Cicadellids of the subfamily Cicadellinae were collected by sweep net and yellow sticky traps, but this taxonomic group was not considered in the current study because it feeds on plant xylem (Miranda et al. 2009a), making its participation in the dissemination of strictly phloem pathogens highly unlikely. The occurrence of species of Cicadellinae in orange orchards has already been studied in previous surveys, mainly in the search for vectors of the bacterium *Xylella fastidiosa* Wells et al. (Paiva et al. 1996, Yamamoto and Gravena 2000, Ott et al. 2006, Nunes et al. 2007, Giustolin et al. 2009, Miranda et al. 2009b).

The number of leafhopper species collected in yellow sticky traps in both sweet orange plots was similar to that collected by sweep net (Figs. 1 and 2; Table 1). The exception was *Bahita infuscata* Osborn (Deltocephalinae), collected only with yellow sticky traps.

The leafhopper data collected from the sticky cards positioned at different heights in the orange trees shows that cicadellids of the Deltocephalinae and Agalliinae subfamilies were mainly captured at a height of 0.3 m in both plots (Figs. 1 and 2). In plot A, the species *A. albidula* was mostly collected from the lower tree branches ($t = 7.2$, GL = 25, $P < 0.05$), as also were *B. hebe* ($t = 6.3$, GL = 24.6, $P < 0.05$), *Chlorotettix minimus* Baker ($t = 2.1$, GL = 41.5, $P < 0.05$), *P. flavicosta* ($t = 3.1$, GL = 34, $P < 0.05$), *S. marginelineatus* ($t = 5.6$, GL = 39.3, $P < 0.05$), and *Protalebrella brasiliensis* Baker ($t = 3.5$, GL = 33.2, $P < 0.05$) (Fig. 1). *B. infuscata* ($t = -1.3$, GL = 35.8, $P = 0.19$), *Curtara samera* DeLong & Freytag ($t = -0.7$, GL = 35.6, $P = 0.5$) and *T. orizicola* ($t = 0.7$, GL = 36.9, $P = 0.5$) showed no differences regarding capture height. In plot B, the results were a bit different but showed similar tendencies. The leafhoppers *B. hebe* ($t = 3.6$, GL = 33.1, $P < 0.05$), *S. marginelineatus* ($t = 4.5$, GL = 30.9, $P < 0.05$), and *P. brasiliensis* ($t = 3.2$, GL = 27.9, $P < 0.05$) were also the principal species

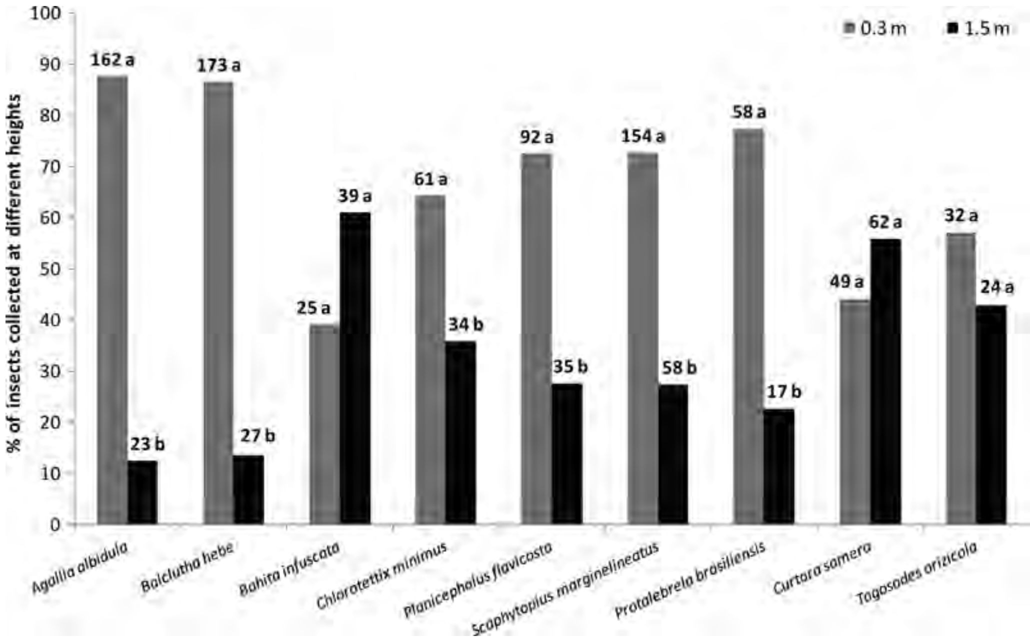

Fig. 1. Distribution of leafhopper species at two heights in the canopy of sweet orange trees in plot A, Nova Granada–São Paulo. Numbers above bars represent the sum of the specimens captured in 22 fortnightly collections with sticky yellow cards (20 cards per height per collection) between 20 April 2008 and 20 May 2009. Percentage values (bars) for the same species and with the same letter are not different according to Student's t-test ($P < 0.05$).

captured in the lower tree sections and *B. infuscata* ($t = -1.7$, GL = 33.4, $P = 0.09$), *C. samera* ($t = -0.2$, GL = 40.9, $P = 0.88$), and *T. orizicola* ($t = 0.9$, GL = 41.8, $P = 0.37$) maintained their homogeneous distribution at both heights. However, although more individuals of the species *A. albidula* ($t = 1.9$, GL = 34.9,

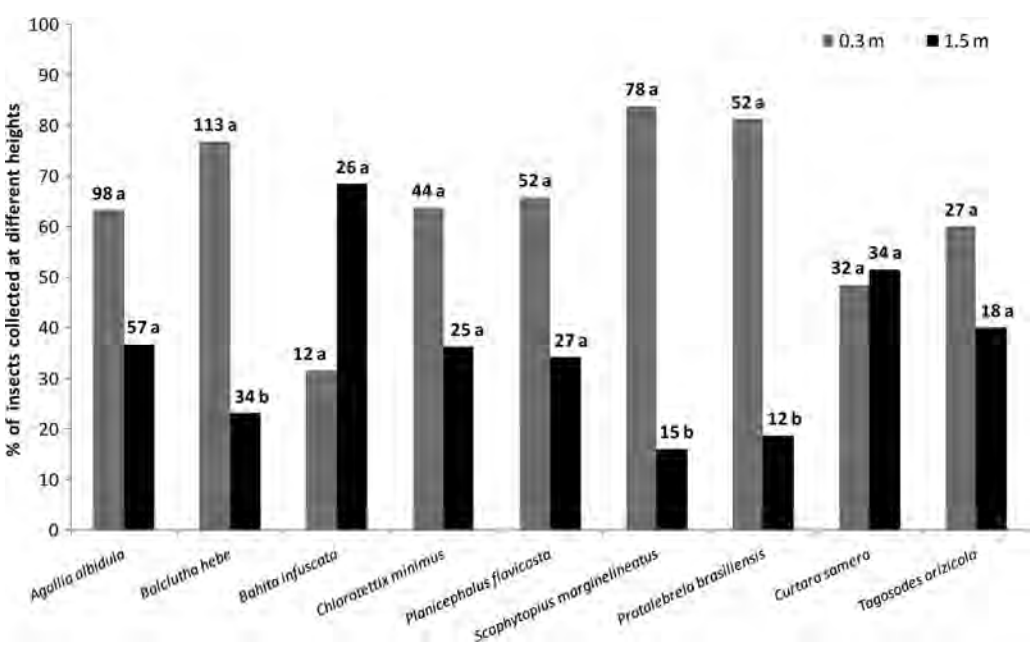

Fig. 2. Distribution of leafhopper species at two heights in the crown of sweet orange trees in plot B, Nova Granada–São Paulo. Numbers above bars represent the sum of the specimens captured in 22 fortnightly collections with sticky yellow cards (20 cards per height per collection) between 20 April 2008 and 20 May 2009. Percentage values (bars) for the same species and with the same letter are not different according to Student's t-test ($P < 0.05$).

Table 2. Faunistic analysis of leafhopper species captured with a sticky yellow trap in sweet orange orchards, 14 and 8 yr old (plots A and B, respectively), Nova Granada–São Paulo, between 20 April 2008 and 20 May 2009

Family, subfamily	Plot A				Plot B			
	D[a]	A[b]	F[c]	C[d]	D	A	F	C
Cicadellidae, Agallinae								
Agallia albidula	D	ha	hf	w	D	ha	hf	w
Cicadellidae, Deltocephalinae								
Balclutha hebe	D	ha	hf	w	D	ha	hf	w
Bahita infuscata	D	r	lf	w	D	r	lf	w
Chlorotettix minimus	D	c	f	w	D	c	f	w
Planicephalus flavicosta	D	c	f	w	D	c	f	w
Scaphytopius marginelineatus	D	ha	hf	w	D	c	f	w
Cicadellidae, Typhlocibinae								
Protalebrella brasiliensis	D	d	lf	w	D	c	f	w
Cicadellidae, Gyponinae								
Curtara samera	D	c	f	w	D	c	f	w
Delphacidae								
Tagosodes orizicola	D	r	lf	w	D	r	lf	w

[a] Dominance: D, dominant; ND, not dominant.
[b] Abundance: r, rare; d, dispersed; c, common; ha, high abundance.
[c] Frequency: lf, low frequent; f, frequent; hf, high frequent.
[d] Constancy: w, constant; y, accessory; z, accidental.

$P = 0.07$), *C. minimus* ($t = 1.3$, GL = 31.8, $P = 0.21$) and *P. flavicosta* ($t = 1.7$, GL = 32.8, $P = 0.09$), were captured in the lower branches, there was no statistical difference between capture heights.

The distribution of the phytoplasma in the citrus trees should be investigated. The potential leafhopper vectors mostly captured at 0.3 m, abundant on the low-lying orchard vegetation, or both, such as *A. albidula, B. hebe, C. minimus, P. flavicosta, P. brasiliensis,* and *S. marginelineatus,* could play a significant role in disseminating this pathogen, if a greater phytoplasma distribution is detected in the lower branches of the citrus canopy.

After the faunistic analysis of the species collected by sticky traps, it was possible to determine which species were predominant in the plots (Table 2). Predominant species are those that show the highest indices of dominance, abundance, frequency, and constancy (Silveira Neto et al. 1995). In plot A, the species *A. albidula, B. hebe,* and *S. marginelineatus* were predominant, but only *B. hebe* and *A. albidula* showed this characteristic in plot B. The species *S. marginelineatus* was classified as being very abundant and very frequent in plot A but only as common and frequent in plot B. However, *P. brasiliensis* was common and frequent in plot B but dispersed and not very frequent in plot A. Despite the variations in abundance and frequency, all the leafhopper species analyzed were constant and dominant in both sweet orange plots (Table 2). Differences in orchard age and the floristic composition of the interrow vegetation or in neighboring areas may have influenced the small variations observed for the faunistic analysis (Table 3) and the relative leafhopper distributions at both capture heights (Figs. 1 and 2).

Table 3. List of species and floristic parameters of invasive plants present in low-lying vegetation in 14- and 8-yr-old (plots A and B, respectively) sweet orange orchards, Nova Granada–São Paulo, February 2009

Family, plant species	Life cycle[a]	Value of IVI[b]	
		Plot A	Plot B
Amaranthaceae			
Alternanthera tenella Colla	Perennial	93.8	69.0
Asteraceae			
Conyza bonariensis (L.) Cronq.	Annual	25.9	NF[c]
Tridax procumbens L.	Annual or biennial	NF	36.3
Commelinaceae			
Commelina sp.	Annual or perennial	12.2	23.3
Euphorbiaceae			
Chamaesyce hirta (L.) Milisp.	Annual	NF	20.4
Malvaceae			
Sida rhombifolia L.	Perennial	102.3	6.3
Poaceae			
Brachiaria plantaginea (Link) Hitchc.	Annual	43.8	44.6
Cenchrus echinatus L.	Annual	NF	11.1
Digitaria horizontalis Willd.	Annual	8.3	22.1
Panicum maximum Jacq.	Perennial	13.7	58.6

[a] Kissmann and Groth (2000).
[b] Importance Value Index (Mueller-Dombois and Ellenberg 1974).
[c] NF, not found.

A larger number of specimens were captured by both the sweep net and the sticky yellow trap in plot A. Four times more insects were collected by sweep net in plot A (1,951:480) and the number was almost twice as high (1,125:756) for the sticky trap. The floristic composition of plot A consisted of more perennial weeds, such *Sida rhombifolia* L., *Panicum maximum* Jacq., and *Althernantera tenella* Colla, plants that can provide shelter, food, or both for the leafhoppers during dry periods.

An additional consideration that must be done regard to the neighboring areas of each plot. Plot A was located at the limits of a small forest of stationary type shrubs, as well as bordering other orchards, whereas plot B was surrounded by other orange orchards and also a rubber plantation. The topographic location of the plots also was different because plot A was in a low-lying area that may have encouraged greater growth of invasive plants and plot B in a higher altitude area. Giustolin et al. (2009) demonstrated that shrub forests can act as a reservoir for leafhoppers found in sweet orange orchards.

Previous surveys in orange orchards have detected species of *Scaphytopius* as predominant (Yamamoto and Gravena 2000, Miranda et al. 2009b). Giustolin et al. (2009) found that the specie *S. marginelineatus* had high faunistic indices (dominant, high abundance, and constant) and was considered important in citrus groves. Miranda et al. (2009b) also showed that insects belonging to this genus are predominant in citrus-growing areas. Paiva et al. (1996) collected high numbers of Deltocephalinae in citrus orchards, mainly *B. infuscata* and *Scaphytopius* sp., on both orange plants and weeds.

Yamamoto and Gravena (2000) captured large numbers of the Thyphlocybinae species *P. brasiliensis* on invasive plants in citrus orchards by using a suction trap, but only a few individuals on orange trees. The collections from different heights corroborate these results because many individuals of this leafhopper species were captured at 0.3 m. Despite the abundance of *P. brasiliensis*, this species is more likely to be a mesophyll feeder as most thyphlocybine leafhoppers; thus, it is less likely to transmit phytoplasmas. However, there are reports of *Empoasca* spp. (Thyphlocybinae) as vectors of phytoplasmas (Pérez et al. 2010, Galetto et al. 2011).

The absence of *B. infuscata* in the sweep net samples suggests that this deltocephalid has no host plants in the low-lying orchard vegetation. However, *B. infuscata* was one of the most frequently caught hoppers in the sticky traps placed at 1.5 m, suggesting more activity of this species in orange tree canopies, possibly feeding on the orange tree or only passing through the orchard in a dispersal movement. Yamamoto and Gravena (2000) captured only a few examples of this species on invasive plants, and in some areas it was found only on orange trees.

Specimen samples of all the leafhoppers collected with the sweep net on the low-lying vegetation growing between sweet orange tree rows (plots A and B) were evaluated by nested PCR for the presence of citrus phytoplasma (16SrIX group). Of 180 samples tested (groups of 10 specimens per sample), seven showed positive result: *B. hebe* (one sample), *S. marginelineatus* (three samples), and *P. flavicosta* (three samples). The identity of the products obtained in the PCR was confirmed by sequencing, with only *S. marginelineatus* being confirmed as a carrier of HLB-phytoplasma, where the sequence *consensus* (793 bp) showed 98% identity with the corresponding sequence of the phytoplasma associated with citrus HLB (accession EU266074.1). These results also were confirmed through cloning of the fragments obtained in the PCR, obtaining a final *consensus* sequence of 856 bp. The analysis of this sequence with BLAST (http://www.ncbi.nlm.nih.gov/BLAST) showed 98% identity with the phytoplasma associated with citrus HLB.

In the literature, leafhoppers of the genus *Scaphytopius* are classified as vectors of >10 plant pathogens (Nielson 1979, 1985). The citrus disease known as "Stubborn," caused by the bacterium *Spiroplasma citri*, and which could be introduced into Brazil, also is transmitted by leafhoppers of this genus. Eckstein (2010) identified a phytoplasma of the 16SrIII group in leafhoppers of the species *Scaphytopius fuliginosus* (Osborn), present in areas affected by the phytoplasma associated with broccoli stunt. This information reinforces the possible action of these insects in disseminating phytoplasmas.

The participation of other insects in phytoplasma dissemination cannot be ruled out. Other cicadellid species identified as common or very abundant in the current study should be investigated, especially those belonging to the Deltocephalinae, that constitute most of the phytoplasma vector species (Nielson 1985, Weintraub and Beanland 2006). The presence of phytoplasmas has already been detected in some species of Deltocephalinae in brassica crops in Brazil, including *B. hebe*, *P. flavicosta*, and *Atanus nitidus* (Linnavuori) (Eckstein 2010, Rapussi-da-Silva 2010).

A preliminary survey of weeds detected 14 species belonging to eight botanical families in the spontaneous vegetation present between rows and beneath the citrus trees canopies in the two plots sampled. The following species were found: *Amaranthus* sp., *A. tenella* (Amaranthaceae), *Bidens pilosa* L., *Conyza bonariensis* (L.) Cronq., *Tridax procumbens* L. (Asteraceae), *Commelina* sp. (Commelinaceae), *Chamaesyce hirta* (L.) Milisp. (Euphorbiaceae), *S. rhombifolia* (Malvaceae), *Brachiaria plantaginea* (Link) Hitchc., *Cenchrus echinatus* L., *Digitaria horizontalis* Willd., *P. maximum* (Poaceae), *Portulaca oleracea* L. (Portulacaceae), and *Spermacoce latifolia* Aubl. (Rubiaceae). In this survey, all the species present were considered, independently of their numbers. The Asteraceae and Poaceae families had the highest number of species in both areas. The floristic analysis determined the important species in each area and some plants detected in the preliminary survey were not considered in the analysis due to their low numbers.

Weeds from five distinct botanical families were found in plot A (Table 3). The most important species were *S. rhombifolia*, *A. tenella*, and *B. plantaginea*, with

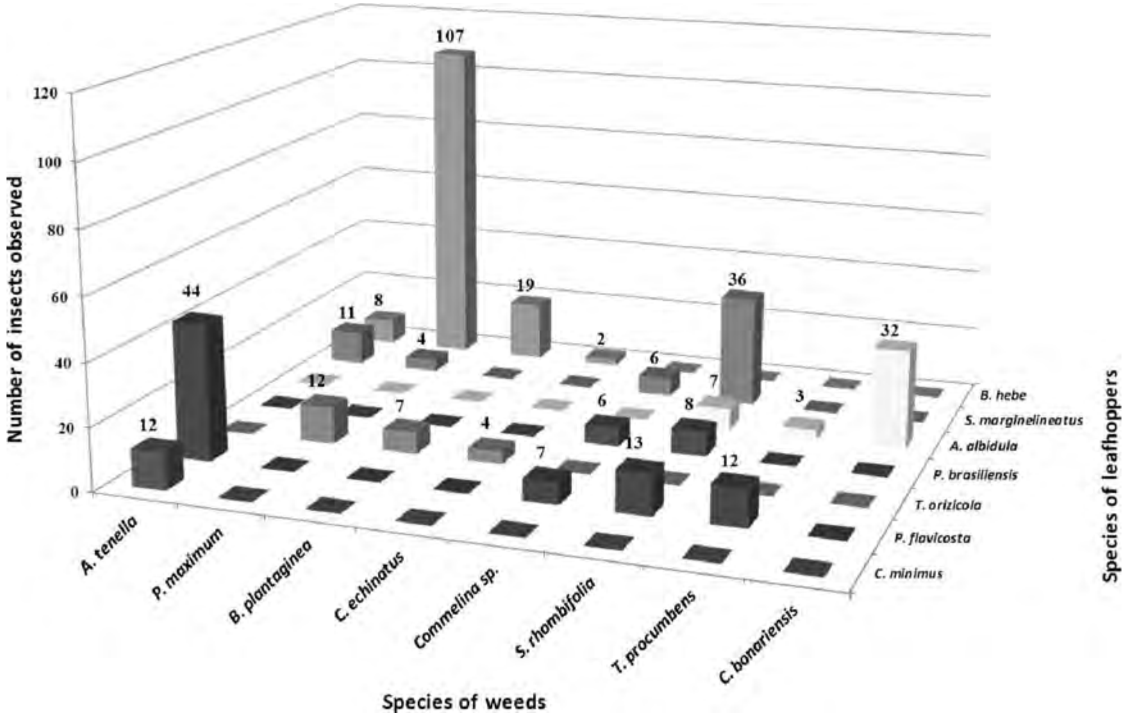

Fig. 3. Total number of individuals of different leafhopper species (*B. hebe*, *S. marginelineatus*, *A. albidula*, *P. brasiliensis*, *T. orizicola*, *P. flavicosta*, and *C. minimus*) observed on plant species of ground vegetation (*A. tenella*, *P. maximum*, *B. plantaginea*, *C. echinatus*, *Commelina* sp., *S. rhombifolia*, *T. procumbens*, and *C. bonariensis* between sweet orange orchard rows (plots A and B), Nova Granada–São Paulo.

an IVI higher than 40. Of these three species, two are perennials (*S. rhombifolia* and *A. tenella*), an important characteristic for the local agroecosystem, principally in the winter, when most annual invasive plant species are unavailable. However, the presence of other plants guarantees greater food diversity for the leafhopper community, which generally contains oligophagous and polyphagous species (Weintraub and Beanland 2006).

In plot B, the variation in IVI values among the plant species was lower than in plot A (Table 3). There was a greater diversity of invasive plants in this area, with 10 plant species belonging to six botanical families. However, the participation of perennials in this area was much smaller than in plot A, principally regarding the species *S. rhombifolia* and *A. tenella.* In plot A, the high IVI value of *S. rhombifolia* (102.3) demonstrates how important this species is in the composition of this area, in contrast to plot B where the species had one of the lowest IVI values (only 6.4).

Most of the invasive plant species found in the floristic survey during the current study are considered important in orange orchards (Carvalho et al. 2005), with the exception of *A. tenella*, *T. procumbens*, and *C. hirta.* This similarity of weed species found in the sample areas with those often verified in orange groves guarantees that the occurrence of these plants was not an isolated case and that this same situation may be found elsewhere.

According to the analysis of association from the chi-square test, the leafhoppers are strongly associated with certain invasive plant species present between the sweet orange tree rows ($\chi^2 = 778.1$, GL = 42, $P < 0.01$). The leafhopper *S. marginelineatus* is associated with plants of *S. rhombifolia, A. tenella, P. maximum,* and *Commelina* sp., being more abundant on the first species (Fig. 3). *B. hebe* is associated with plants of *A. tenella, B. plantaginea, Commelina* sp., and mostly with *P. maximum,* where it stood out owing to the high number of specimens found. The species *P. flavicosta* was associated with the plants *A. tenella, S. rhombifolia, T. procumbens,* and *Commelina* sp, being found in higher numbers on the first species. Another deltocephaline, *C. minimus*, was only observed on *A. tenella.* The leafhopper *A. albidula* was associated with the species *S. rhombifolia, T. procumbens,* and *C. bonariensis,* especially with the latter species. The only hopper that did not belong to the Cicadellidae, *T. orizicola* (Hemiptera: Delphacidae), was found on plants of the Poaceae, including *P. maximum, B. plantaginea,* and *C. echnatus.*

These data corroborate those from insect captures made in the different areas, together with the data on the floristic composition of spontaneous orchard vegetation. The leafhopper *S. marginelineatus* was more abundant and frequent in plot A (Tables 1 and 2), where the plant species *S. rhombifolia* is more important (Table 3). This information was reinforced with the data of association between leafhoppers and host plants, where *S. rhombifolia* is the main host of *S. marginelineatus* among the plants sampled. The same is true for *A. albidula*, which had higher capture in plot A, with a high IVI of the invasive plant species *C. bonariensis*, main host of this leafhopper for the conditions of the current study. This leafhopper frequents several host plants from a wide variety of taxonomic groups. Besides being a vector of a tomato virus, it has other hosts, including lettuce (*Lactuca sativa* L.), cotton (*Gossypium hirsutum* L.), potato (*Solanum* spp.), beet (*Betula vulgaris* L.), chicory (*Cichorium intybus* L.), spinach (*Spinacia oleracea* L.), dry bean (*Phaseolus* spp.), tobacco (*Nicotinia* spp.), sunflower (*Helianthus annuus* L.), flax (*Linaria vulgaris* Mill.), papaya (*Carica papaya* L.), bell pepper (*Capsicum* spp.), and wheat (*Triticum aestivum* L.) (Silva et al. 1968).

Leafhoppers have a wide variety of hosts (Nielson 1985). Surveys in citrus orchards demonstrate that the quantity and diversity of species are influenced by the collection site (Paiva et al. 1996, Yamamoto and Gravena 2000, Giustolin et al. 2009). Generally, the deltocephalines and agalliines are found on invasive plants in crops, and the type of plant species in the areas can alter the number of insects collected. Yamamoto and Gravena (2000) collected many specimens of the leafhoppers *P. brasiliensis*, *P. flavicosta*, and *C. minimus* when they surveyed plant infestations at the site. Lopes and Oliveira (2004) collected specimens of *B. hebe* on vegetation growing next to a corn crop. However, these studies did not conduct a floristic survey of the invading plants to determine the most important species in each area. Leafhoppers of this genus feed on grasses but there are few reports of the most important plant species for their development (Wilson and Claridge 1991).

The delphacid *T. orizicola* is reported as grasses insect pest of agricultural importance such as rice, *Oryza sativa* L., also developing on grass weeds in this crop, such as *Echinochloa* sp. (Wilson and Claridge 1991).

Studies that involve surveys of leafhoppers, weeds, and the detection of pathogens in insects are uncommon in the Brazilian literature. The information from the present research will serve as basis for studies on orchard weed management with the aim of reducing disease incidence in plants.

Acknowledgments

We thank Keti Zanol for taxonomic identification of the hopper specimens; Fischer S.A. group for conceding the experimental area; Fundecitrus for technical and financial support, and Coordination for the Improvement of Higher Level Personnel (CAPES) and National Council for Scientific and Technological Development (CNPq) for granting a scholarship to the R.N.M.

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Received 2 August 2011; accepted 26 January 2012.