

## Survey for the Asian Citrus Psyllid, *Diaphorina citri*, and Citrus Huanglongbing (Greening Disease) in Texas

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### ABSTRACT

The Asian citrus psyllid, *Diaphorina citri*, one of the vectors of citrus huanglongbing (HLB) or greening, was first recorded in Texas in the Lower Rio Grande Valley in 2001. After the discovery of HLB in Florida in 2005, a USDA-APHIS-PPQ funded survey was conducted in 2006 to detect the presence of HLB and determine the current distribution of the psyllid in all parts of Texas where citrus is grown, both commercially and in residential properties. During this survey, psyllids were found throughout south Texas, with a northern limit from Del Rio in the west, through San Antonio to the coastal area north of Corpus Christi. They were also found on trees in residential properties in Houston and surrounding areas. Psyllids were present on trees in 32 of the 87 counties where citrus is grown. No typical HLB symptoms were observed anywhere, but 309 leaf samples with various yellowing patterns were sent to the USDA-AMS laboratory in Gastonia NC for HLB analysis by PCR. No positives have been detected.

*Additional index words:*

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Citrus Huanglongbing (HLB), commonly known as greening, is a devastating disease which has been endemic in many parts of Asia and Africa for several decades (da Graça, 1991; da Graça & Korsten, 2004; Bové, 2006). The disease is caused by phloem-limited bacteria belonging to the genus *Candidatus Liberibacter*, and is transmitted by two species of citrus psyllids (Hemiptera: Psyllidae). The Asian form of greening, caused by *Ca. L. asiaticus*, and naturally vectored by *Diaphorina citri* Kuwayama, is more serious, causing severe symptoms in all commercial citrus varieties sometimes leading to tree death. The African form, caused by *Ca. L. africanus* and transmitted by *Trioza erytreae* Del Guercio, mainly causes losses in oranges and mandarins and is less severe in hotter climates. A new species discovered in Brazil, *Ca. L. americanus*, appears to cause a disease similar to the Asian form (Teixeira et al., 2005b).

*D. citri* has been present in Brazil for many years (Lima, 1942 cited by Bové, 2006), but was only found in Florida in 1998 (Knapp et al., 1998). Within 3 years, the psyllid was found in Texas (French et al., 2001). In 2004, HLB was confirmed to be present in Brazil (Teixeira et al. 2005a), and in 2005 it was found in Florida (Halbert, 2005). In both locations, it appears that the disease had been there undetected for some time since follow-up surveys showed the disease was already established in several other locations (Ayres et

al., 2005; Gottwald et al., 2006). In Brazil most trees are infected with *Ca. L. americanus*, with the remainder having *Ca. L. asiaticus* (Teixeira et al., 2005b). In Florida, only *Ca. L. asiaticus* has thus far been found (Gottwald et al., 2007).

Since *D. citri* is present in Texas, a statewide survey was conducted in 2006 to determine how widespread the insect had become, and whether any trees with HLB could be detected. Commercial citrus production is concentrated in Hidalgo, Cameron and Willacy counties which are located in the Lower Rio Grande Valley (LRGV) adjacent to the border with Mexico (da Graça & Sauls, 2000). In addition, many citrus trees are grown in residential properties throughout the southern portion of the state, and into east Texas close to the Louisiana border. Some citrus is also grown in containers in other areas where they can be placed under shelter during the winter.

### METHODS

In the three counties of the LRGV, retail and wholesale nurseries, commercial citrus orchards, trailer parks and dooryards were surveyed. Similar surveys were made to nurseries, retail boxstores and dooryards across the state, wherever citrus was reported to be growing. A total of 32 survey trips were made to areas outside the LRGV. Citrus trees of

various varieties were found as far west as El Paso, as far north as Dallas-Fort Worth and along the eastern border with Louisiana. When permission from property owners was obtained, trees were visually inspected for the presence of psyllids and greening-like symptoms. Adult psyllids were collected with aspirators and immediately transferred to 95% ethanol. Young flushes with nymphs were also collected and placed in glass vials containing 95% ethanol, and later collected with a camel hair brush. Leaves with any pattern of yellowing were collected and placed in Ziploc bags, and transported to the Citrus Center in a cooler chest.

In addition, Texas Department of Agriculture (TDA) inspectors who regularly monitor Mexican fruit fly traps on approximately 5,000 sentinel trees in orchards and residential properties in the LRGV were trained by scientists at the Citrus Center to recognize HLB symptoms. Leaf samples from 314 trees (220 from orchards and the remainder from residences) were collected by them and screened by one of the authors (B. Salas) for possible submission for laboratory analysis.

Although no typical symptoms of HLB were found anywhere in the state, a total of 309 leaf samples displaying various yellowing patterns from most areas were sent by overnight courier to the USDA-AMS diagnostic laboratory in Gastonia NC, for PCR analysis for *Ca. L. asiaticus* (Jagoueix et al., 1996; Hocquellet et al., 1999).

### SURVEY RESULTS

A total of 94 counties, grouped into geographical regions (Kingston, 1987) were surveyed (Table 1). Citrus trees were found in 87 of them, and psyllids were identified in 32. The presence/absence of psyllids on citrus throughout the state is presented in Fig. 1.

The Asian citrus psyllid was recorded throughout the southern counties of Texas (Lower Rio Grande Valley, South Texas, Southwest Texas, and Southern coastal regions). The most westerly finds were in Del Rio (Val Verde Co.) and Eagle Pass (Maverick Co.). Several infested trees were found in San Antonio (Bexar Co.) and the one find in the Central Region in Caldwell Co. north east of Bexar Co. was on a potted tree recently purchased in San Antonio. No other finds were recorded in the Central region. In Harris Co. (Houston and surrounding cities), psyllids were recorded at 27 sites. One site south of Houston in Brazoria Co. was found with a single psyllid adult, and two nurseries north east of Houston had psyllids on one tree each; these were possibly new arrivals. No psyllids were found at other sites surveyed along the

Gulf Coast, southeast Texas near the Louisiana border, west Texas nor north Texas.

None of the 309 leaf samples tested positive for *Ca. L. asiaticus*. Locations from which samples were collected for analysis are shown in Table 1.

### DISCUSSION

The extent of citrus presence in residential yards was a surprise, but could be explained by the mild winters experienced in Texas in recent years and the increasing numbers of citrus enthusiasts in various cities. For example, El Paso and Fort Worth are not usually considered suitable for citrus, but planted trees were found in sheltered locations. In areas where winter cold would damage trees, most trees were in containers which are moved into protected areas during winter. Large wholesale nurseries are in operation at several locations in east and southeast Texas, and in recent years there have been increased orders of virus-free budwood from these nurseries to the Texas budwood certification program (Kahlke et al., 2005).

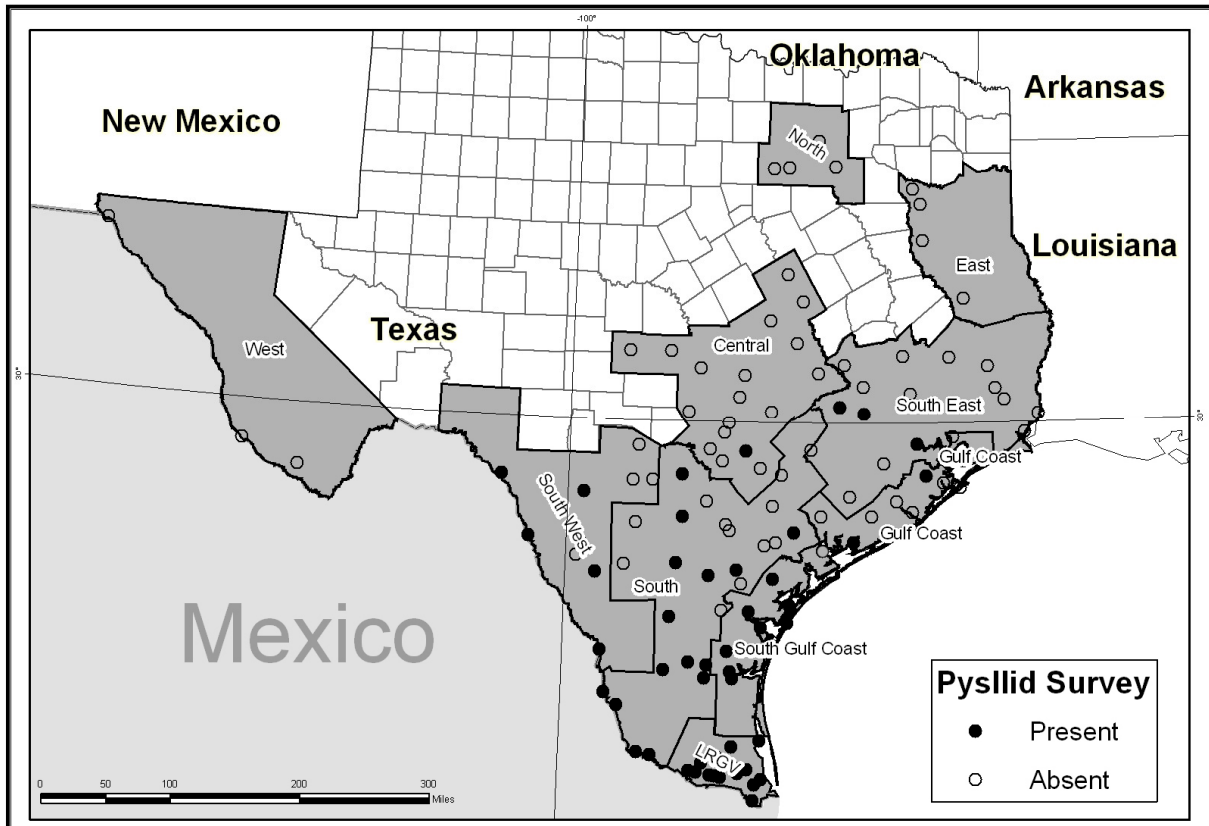
Since its first record in Texas in 2001 (French et al., 2001), the Asian citrus psyllid, *D. citri*, has spread and can be found throughout south Texas from Del Rio in the west, to San Antonio in the center, and Palacios near the Gulf Coast. In addition, psyllids were present at several sites in Houston, and were also found in two nurseries to the north-west. It will be important to determine if psyllids can overwinter in all the sites where they were found, and if there is further spread to other areas in the future. No psyllids were found in the south east Texas counties of Jefferson, Orange, Hardin, Tyler, where there is a significant number of citrus trees. Reports from Louisiana also indicate that psyllids have not been recorded there yet (B. Castro, J. Boudreaux, pers. comms.).

In addition to citrus plants, psyllids infest other rutaceous plants such as orange jasmine (*Murraya* spp.), one of the preferred hosts (Halbert and Manjunath, 2004). It is possible that the psyllid was brought into Texas from Florida on orange jasmine because of the high volume of imports from Florida of this plant by Texas retail nurseries. It is also possible that it was introduced from Mexico, where it was first recorded in 2002 (Lopez-Arroyo et al., 2005), although it could have been present there before then. During the survey, orange jasmine plants were found in nurseries in the LRGV, and in Corpus Christi, but not elsewhere in Texas. It is possible that psyllids moved from the LRGV to other counties in the state on potted citrus trees; the observation that psyllids were found at sites near major highways may be significant information.

**Table 1.** Results of surveys for Asian citrus psyllid, *Diaphorina citri*, and Citrus Huanglongbing (HLB) in Texas (2006)

Region <sup>a</sup>	No. counties with psyllids/ no. surveyed	Site type <sup>b</sup>	No. sites surveyed/ no. with psyllids	No. leaf samples assayed <sup>c</sup>
Lower Rio Grande Valley	3/3	G	73/51	102
		R	12/10	19
		N	2/2	4
South	12/20	G	2/2	1
		R	23/17	25
		N	8/1	3
		BG	1/1	1
Southwest	5/8	G	4/3	2
		R	7/4	2
		N	4/1	6
Southern coast	6/6	R	24/19	17
		N	2/1	3
Gulf Coast	2/6	G	1/0	1
		R	15/3	18
		N	2/0	0
Central	1/16	R	12/1	3
		N	7/0	3
		RB	11/0	10
Southeast	3/18	G	5/0	12
		R	30/8	25
		N	15/4	18
		RB	3/0	2
East	0/3	N	5/0	9
		RB	1/0	1
North	0/4	R	1/0	0
		N	4/0	3
		RB	2/0	2
		BG	1/0	5
West	0/3	R	10/0	11
		N	1/0	1
TOTALS	32/87		288/127	309 <sup>b</sup>

<sup>a</sup> see Kingston (1987)<sup>b</sup> G=Grove; R=Residential; N=Nursery; RB= Retail Boxstore; BG=Botanic Garden<sup>c</sup> PCR assays performed by USDA-AMS laboratory, Gastonia NC. All were negative for HLB



**Fig. 1.** Map of Texas showing locations of citrus trees surveyed for psyllids and huanglongbing in 2006. The counties where citrus was found are grouped into geographical regions as described by Kingston (1987).

The fact that no HLB-like symptoms were observed, and no confirmed positive laboratory results were obtained, is encouraging. However, the experience in Florida where, despite surveys, HLB was not found for several years (Halbert, 2005), is a concern for Texas. The long latent period (Zhao, 1981) means that any tree possibly infected in the past 1-2 yrs may not have developed symptoms by the time of this survey. Continued surveillance is essential in commercial citrus orchards and in dooryards across the state.

In addition, it is important to develop and implement aggressive management strategies for the Asian psyllid in order to substantially reduce vector populations. Florida has introduced new regulations for its citrus budwood program, which require all operations be conducted in insect-resistant structures (Crawford, 2007). The disease-free germplasm for the Texas citrus industry is now being established in similar structures.

Several laboratories are attempting to develop

more sensitive diagnostic assays for HLB to enable earlier detection of the disease. Real time PCR is more sensitive than the PCR assays used for samples in this survey, and it is being assessed for use on leaf extracts (Irey et al., 2006) and psyllids for both qualitative and quantitative assays (Manjunath et al., 2006). HLB has always been detected in new areas some time after introduction, with the result that it rapidly becomes endemic, with resulting economic losses. If HLB is introduced into Texas, and can be detected at an early stage before becoming widespread, there may be an opportunity of implementing a successful eradication program and avoiding serious crop losses.

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**Notes added in proof:** (1) Surveys continued through 2007. Psyllids were not found on citrus in any additional counties, and *Ca. L. asiaticus* was not detected in any of the 238 leaf samples from around the state sent to the USDA-AMS laboratory in Gastonia NC.

(2) In May 2008, *Diaphorina citri* was detected for the first time in Louisiana (S. W. Kuehn, USDA-APHIS-PPQ, pers.comm.)

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