

SCIENTIFIC NOTE**FIRST REPORT OF INVASIVE *Diaphorina citri* (HEMIPTERA: LIVIIDAE) AND ITS ASSOCIATED PARASITOID *Tamarixia radiata* (HYMENOPTERA: EULOPHIDAE) IN THE ESPÍRITO SANTO STATE, BRAZIL**

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ABSTRACT- Invasive species are responsible to cause several problems for both environment and managed systems, such as agriculture. The main citrus pest insect worldwide is the invasive species Asian citrus psyllid *Diaphorina citri* (Hemiptera: Liviidae), a vector of the 'Candidatus Liberibacter spp.' that causes the citrus disease known as greening or Huanglongbing (HLB). This insect vector was found widespread in Espírito Santo State, Brazil, and *Tamarixia radiata* (Hymenoptera: Eulophidae) was a parasitoid associated with its nymphs. In this study, we report the range expansion of this insect vector in Brazil, and discuss some measures to avoid the HLB disease entrance in the state, and *D. citri* populations' management.

KEYWORDS: Asian citrus psyllids. Invasive species. Insect vector. Huanglongbing.

RESUMO- Espécies invasoras são responsáveis por causar vários problemas tanto para o meio ambiente quanto para os sistemas manejados, como a agricultura. O principal inseto praga de citros em todo o mundo é a espécie invasora, *Diaphorina citri* (Hemiptera: Liviidae), psílídeo vetor da bactéria "Candidatus Liberibacter spp.", agente etiológico da doença cítrica conhecida como greening ou Huanglongbing (HLB). Este inseto vetor foi encontrado de forma generalizada no Espírito Santo, Brasil. O parasitoide *Tamarixia radiata* (Hymenoptera: Eulophidae) também foi encontrado associado às ninfas de *D. citri*. Neste estudo, relatamos o grande alcance desse inseto vetor no Brasil e discutimos algumas medidas para evitar a entrada da doença HLB no estado, bem como o manejo das populações de *D. citri*.

PALAVRAS-CHAVE: Psílídeo. Espécie invasora. Inseto vetor. Huanglongbing.

Invasive species are one of mankind's greatest challenges, which cause several problems in natural and managed ecosystems, including agricultural where economic losses can be severe (BENNETT, 2013). Biological invasions can cause new species interactions, for example between plants, pathogens, and insects vectors (RICHARDSON et al. 2000). Currently, the invasive species Asian citrus psyllid (*Diaphorina citri*) Kuwayama (Hemiptera: Liviidae) is considered the main citrus pest in the world (HALL et al. 2013). This species is able to transmit the 'Candidatus Liberibacter spp.', an endogenous and phloem-limited bacterium, which causes a destructive citrus disease previously known as greening and nowadays as Huanglongbing (HLB) (HALBERT and MANJUNATH, 2004; BOVÉ 2006). Furthermore, *D. citri* is also able to transmit plant pathogen 'Candidatus Phytoplasma aurantifolia', the etiological agent of Witches' Broom Disease of Lime (WBDL) (QUEIROZ et al., 2016). *Diaphorina citri* was reported for the first time in Brazil at least 70 years ago (LIMA, 1942) and it has a wide range of hosts, particularly plant species belong to the family Rutaceae (HALBERT and MANJUNATH, 2004).

Until recently, the American continent was HLB disease free, but in March 2004 the disease symptoms were observed in the São Paulo state, Brazil, and in August 2005 in Florida,

USA, two of the main citrus producing regions in the world (BOVÉ, 2006). The typical HBL symptoms are yellow shoots, small and lopsided fruits, strong color inversion, aborted seeds, by which were observed in Brazil in sweet orange trees (COLETTA-FILHO et al., 2004; AYRES et al., 2005; TEIXEIRA et al., 2005). HLB represents a threat to world production of citrus, particularly where *D. citri* is widespread. This disease is related in Brazilian citrus producing-regions in the states of Minas Gerais, Paraná, and São Paulo. However, the Asian citrus psyllid has been reported on several Brazilian states, such as Amazonas, Bahia, Ceará, Pará, Pernambuco and Roraima (SILVA et al. 1968; MARSARO JÚNIOR et al. 2014). Here, we are reporting the occurrence of the invasive species *D. citri* for the first time in the Espírito Santo state.

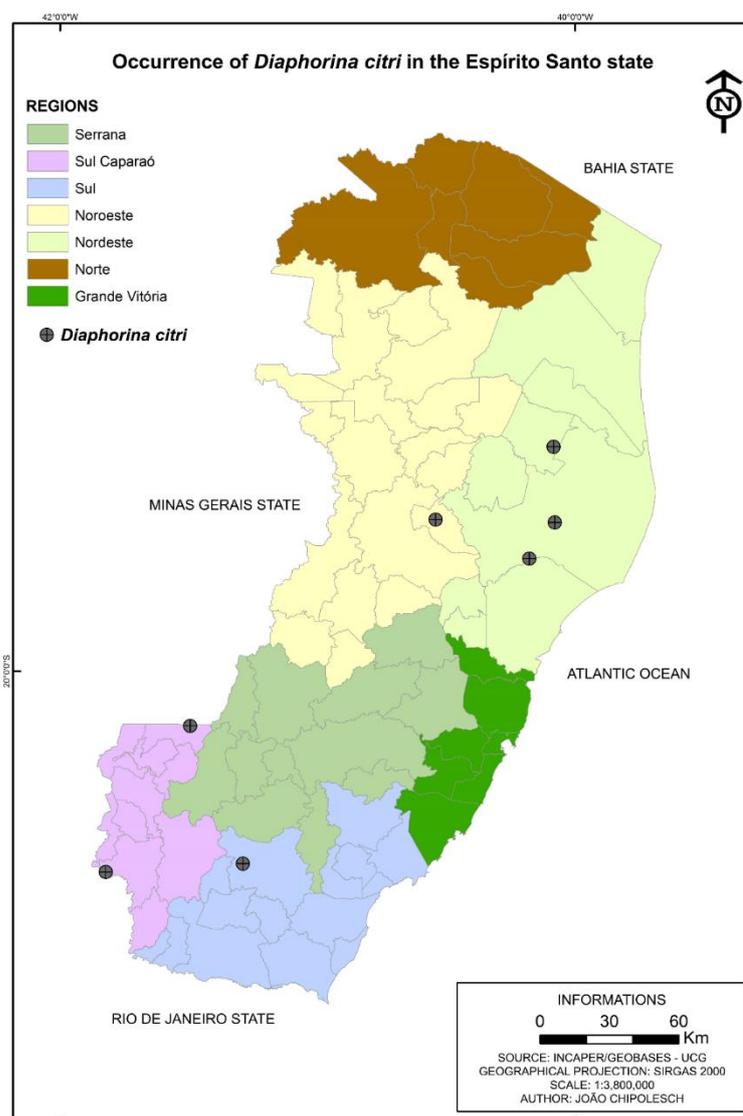
Adults, nymphs and eggs of *Diaphorina citri* were collected in seven different places in the Espírito Santo state (Figure 1). In the Northern, it was found in the Research Experimental Station of Incaper (19°25'01.5"S 40°04'44.8"W), and Santa Luzia Farm in the municipality of Linhares (19°33'30.6"S 40°10'40.0"W); Research Experimental Station of Incaper (Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural) in Sooretama (19°07'14.8"S 40°05'01.5"W), Marilândia (19°24'20.4"S 40°32'30.2"W), and Cachoeiro do Itapemirim (20°45'12.0"S 41°17'25.6"W). In Southern *D. citri* was found in the municipalities of Ibatiba (20°12'49.23"S 41°29'44.07"W) and Guaçuí (20°47'8.68"S 41°49'24.23"W) (Figure 2). Samples were collected in the canopies of citrus (*Citrus sinensis*), and orange jasmine (*Murraya paniculata*), and associated with shoots and leaves. All the samples were infested with *D. citri*. Nymphs of *D. citri* were placed in plastic pots with fresh shoots of *M. paniculata* to observe parasitoid emergence (Figure 1), which about 50% of the nymphs had parasitoid emergence. Parasitoids emerged were preserved on 70% alcohol and then send to species identification by Dr. Valmir Antonio Costa from the Biological Institute of the Department of Agriculture and Supply of the São Paulo State.

Figure 1. *Diaphorina citri* (Hemiptera: Liviidae) eggs (A), nymph (B), and adult (C) on shoots of the orange jasmine (*Murraya paniculata*) plants at Research Experimental Station of Incaper in Linhares, Espírito Santo state, Brazil. Detail of the parasitized nymphs by *Tamarixia radiata* (Hymenoptera: Eulophidae) (D). March 2015.





Figure 2. Occurrence of *Diaphorina citri* in the Espírito Santo state. The different colors represent the main regions in the Espírito Santo state. Gray circle represents the places where *D. citri* were collected.



Diaphorina citri may be considered widespread in Espírito Santo state. On the other hand, this state is still free of HLB disease. Therefore, this situation needs more attention since citrus growing is one of the major agricultural products in this State, mainly cropped by small farmers (IBGE, 2013). The Espírito Santo state produced around 56,000 tons in 2014 (INCAPER, 2015). Due to its importance to citrus production, quarantine measures are extremely important to avoid the disease entrance, such as avoid the transit of citrus seedlings because *D. citri* fly to short distances (SAKAMAKI, 2005). These plants may be infected by ‘*Ca. Liberibacter* spp.’ pathogen or may carry psyllid eggs, nymphs, and adults (PELZ-STELINSKI et al., 2010). Other effective quarantine measures may be the eradication of the ornamental plant *M. paniculata* because it is a preferred host of *D. citri*. Moreover, the HLB disease symptoms may delay to appear, and some PCR techniques cannot detect the pathogen when it is still in low levels on plants (BOVÉ, 2006). Roguing of ‘*Ca. Liberibacter* spp.’ infected plants followed by chemicals is the main control method to *D. citri*. However, chemicals may induce resistant populations (TIWARI et al., 2011) and may reduce natural enemies, which can cause the secondary pest outbreaks (DUTCHER, 2007). Another management measure to avoid the ‘*Ca. Liberibacter* spp.’ spread is acquire citrus seedlings from nurseries protected with anti-aphid screens and seedlings certified by inspection governmental agencies, for example, Coordenadoria de Defesa Agropecuária (CDA) of the São Paulo state (FUNDECITROS, 2017).

Tamarixia radiata (Waterston 1922) (Hymenoptera: Eulophidae) was found associated with *D. citri* nymphs (Figure 1), and this is a potential parasitoid to be used on biological control programs. This is an ectoparasitoid, a single adult can parasitize up to 500 nymphs (CHIEN, 1995; SKELLEY and HOY, 2004) with a parasitism rate > 77% (GÓMEZ-TORRES et al., 2012), and able to attack from third to fifth instars of *D. citri* (MCFARLAND and HOY, 2001; SKELLEY and HOY, 2004). Therefore, this is a parasitoid that should be considered in management programs for this pest in Espírito Santo state, Brazil.

Diaphorina citri was found on elevations higher than 600 m as in Guaçuí and Ibatiba, but in lower populations. However, there is a strong evidence that *D. citri* populations are negatively correlated with elevation, and suggests that the lower abundance of *D. citri* at higher elevations results in lower HLB disease incidence. It may be a consequence of differences in temperature, air pressure, oxygen levels, ultraviolet light, or other factors alone or combined (JENKINS et al., 2015). New studies should be carried out to confirm the correlation between *D. citri* population level and altitude. Since that climate conditions are fundamental to *D. citri* management, particularly to avoid citrus cultivation in lower regions, to produce seedlings in higher altitudes, and therefore to prevent the disease spread by insect vector. Thus, future studies should be directed towards to avoid the HLB disease entrance in the Espírito Santo state, and to *D. citri* populations’ management.

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