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SCIENTIFIC NOTE

Occurrence in Brazil of *Haplaxius crudus* (Hemiptera: Cixiidae), Vector of Coconut Lethal Yellowing

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Abstract

The lethal yellowing (LY) is a major phytoplasma causing disease seriously threatening coconut plantations worldwide, with imminent risk of entering Brazil. The LY phytoplasma is restricted to the phloem and transmitted by the planthopper *Haplaxius crudus* (Van Duzee) (Cixiidae). In this study, Auchenorrhyncha were collected on the leaves of Dwarf vs. Dwarf hybrids and Brazilian Green Dwarf Jiqui in the Brazilian northern state of Para using yellow adhesive traps in May of 2016. The planthopper *H. crudus* was found in coconut plantations of Brazilian Green Dwarf Jiqui, accounting for 87% of the individuals captured. This is the first report of vector *H. crudus* in Brazilian coconut plantations. These findings are of great scientific relevance since *H. crudus* could negatively impact the Brazilian coconut industry and this knowledge could be used in contingency measures in the case of LY be introduced in the country.

The coconut tree (*Cocos nucifera* L.) is a perennial crop of great socioeconomic importance for growing countries in Asia, Africa, Latin America, the Caribbean, and Pacific Islands (Persley 1992) and its consumption is increasing in many countries around the world (Gunn *et al* 2011). Brazil is the fourth largest producer of coconut, with an approximate production of 2.2 million tons per year in a harvested area of 287,000 ha, surpassed by Indonesia, the Philippines, and India, which together represent 78% of the world production (FAO 2017).

Lethal yellowing (LY) is caused by phytoplasmas belonging to the 16SrIV group in the Caribbean and is the most destructive coconut disease in the world (McCoy *et al* 1983, Broschat *et al* 2002). Phytoplasmas colonize the tissues and organs of vector insects and are restricted to the phloem tissues (Purcell 1982; Cousin & Boudon-Padieu 2002). Planthoppers (Fulgoroidea) and the leafhoppers of the subfamily Deltocephalinae are phloem feeders, with several species involved in phytoplasma transmission. The subfamily Deltocephalinae contains the largest number of phytoplasma vectors (Weintraub & Beanland 2006).

LY spreads rapidly and is highly destructive, resulting in the death of coconut trees and around 38 other species of palms within a period of 3 to 6 months after the detection of the first symptoms (Broschat *et al* 2002; Dollet *et al* 2009). Although the injection of the antibiotic oxytetracycline hydrochloride, every 4 months, has been proven effective against LY (McCoy 1975; McCoy *et al* 1976), the costs of this treatment prevent their use (Gurr *et al* 2016). LY in coconut trees has been observed in the USA, Mexico, Jamaica, Cuba, Haiti, Dominican Republic, the Bahamas, Cayman Islands, Antigua and Barbuda, and Saint Kitts and Nevis (Harrison 2012; Gurr *et al* 2016). More recently, LY was observed in Saint Martin and Saint Bartelémy (Dollet M. unpublished data 2013). Lethal yellowing type syndromes (LYTS) also occur in West and East Africa: respectively, Côte d'Ivoire, Ghana, Togo, Nigeria, Cameroon, and Kenya, Tanzania, and

Mozambique (Dollet *et al* 2009; Arocha-Rosete *et al* 2014).

Owing to the proximity of the Northern borders of South America with some of the Caribbean countries affected, LY is at imminent risk of entering Brazil. The inoculum can be introduced accidentally, with any kind of contaminated vegetal material or with insects transported with turf grass and other weeds as nymphs of planthoppers feed on such roots.

The only known LY vector is the planthopper *Haplaxius crudus* (Van Duzee) (Cixiidae); however, other cixiids such as *Nymphocixia caribbea* (Fennah) Emeljanov 2007 could be involved in the transmission of LY phytoplasmas of the group 16Sr DNA IV (Dollet *et al* 2010). The capacity of *H. crudus* to transmit phytoplasmas was first verified by Howard *et al* (1983) in Florida.

The imminent risk of LY introduction in Brazil highlights the urgent need of surveying for this planthopper in coconut plantations. Assessing the occurrence of *H. crudus* could support future contingency measures in the case the disease reaches the country.

Thus, this study aimed to observe the occurrence Auchenorrhyncha associated with coconut plantations of Brazil, with focus on LY vector.

Auchenorrhyncha were collected in two areas using yellow adhesive traps placed in the leaves of coconut plantations in the northern state of Para (Santa Izabel do Para municipality), in the 2016 rainy season (May, 196 mm of rainfall) (Fig 1). The first area (01°14.744′ S, 048°03.533′ W) comprised a 5-year-old field plantation of Dwarf vs. Dwarf hybrids ((1) Brazilian Green Dwarf Jiqui; (2) Yellow Dwarf (Brazilian and Malayan) × Brazilian Green Dwarf Jiqui; (3) Red Dwarf (Brazilian and Malayan) × Brazilian Green Dwarf Jiqui; (4) Cameroon Red Dwarf × Yellow Dwarf (Brazilian and Malayan); (5) Cameroon Red Dwarf × Green Dwarf (Brazilian and Malayan); (6) Cameroon Red Dwarf × Brazilian Green Dwarf Jiqui) (Fig 2a). In the second area (01°12.390′ S, 048°02.665′ W), sampling was conducted in a 6-year-old field plantation in two plot of Brazilian Green Dwarf Jiqui (Fig 2b). Thirty traps (Isca®, double-sided glue, 8.5 × 11 cm) were placed on the leaves of 30 randomly selected trees in each area, with a total of 60 traps.

The traps were collected after 8 days and taken to the laboratory for the removal of Auchenorrhyncha with the aid of a solvent (Tira Cola Allchem®). Specimens were deposited in the Museum Emilio Goeldi and in the Entomological Collection of Embrapa Amazônia Oriental, Para state, Brazil. Subsequently, specimens were sent to the entomological collection of the University of Delaware, Department of Entomology and Ecology of Wild Animals, Newark, Delaware, USA. Dr. Charles R. Bartlett identified specimens at the species level using specialized literature.

For the molecular identification, the total DNA was extracted with Dneasy® Plant Mini Kit (Quiagen), diluted, and then amplified the COI (cytochrome oxidase subunit I) gene

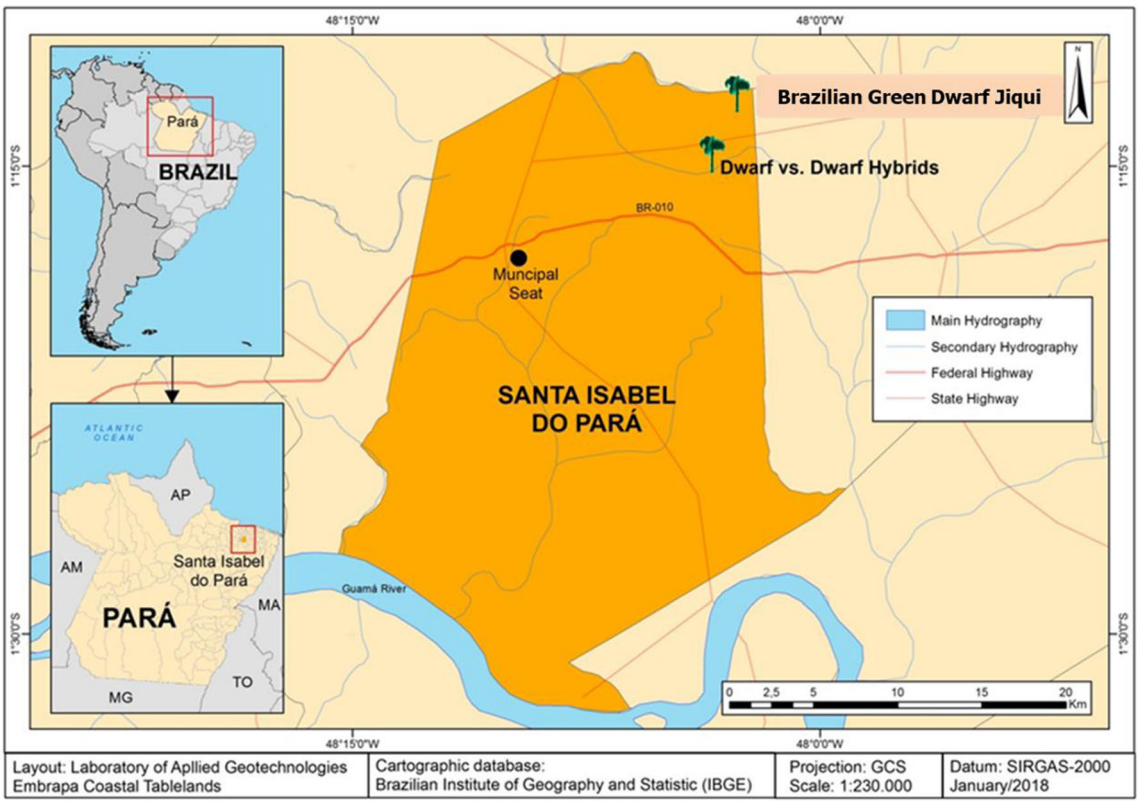

Fig 1 Sampling areas of Auchenorrhyncha in coconut plantations located in the municipality of Santa Izabel do Pará, in Para of state.

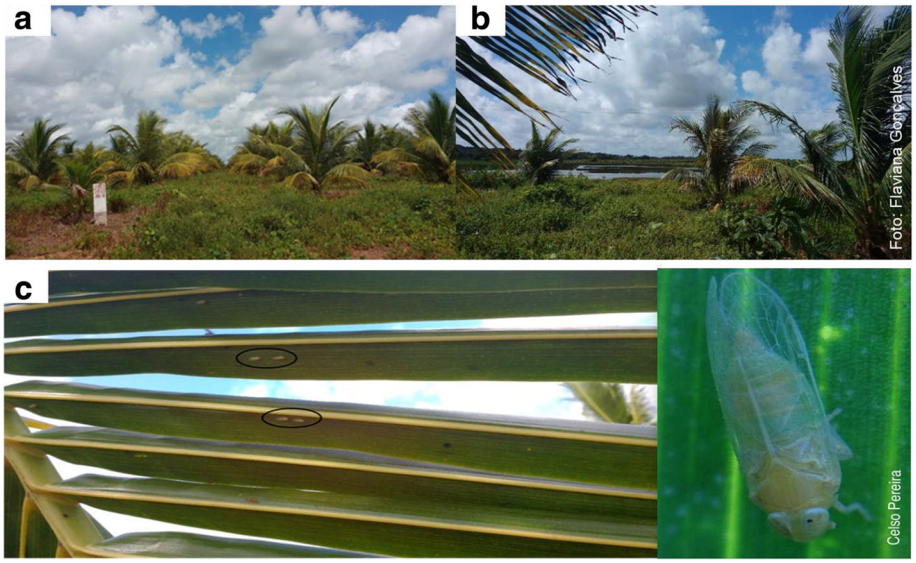

Fig 2 Samplings areas of Auchenorrhyncha in plantations of Dwarf vs. Dwarf hybrids (**a**) Brazilian Green Dwarf Jiqui (**b**), and *Haplaxius crudus* on coconut leaves (**c**) on Santa Izabel do Para, Para

by PCR using the primers 2183 (CAACATTTATTTTGATTTTTTGG) and UEA (8 AAAAATGTTGAGGGAAAAATGTTA) according to Ceotto *et al* (2008). After amplification, the size of the products was confirmed with electrophoresis in 2% agarose gel, stained with ethidium bromide, and visualized under UV light. Then the samples were purified with the GFX™ PCR DNA and Gel Purification kit (GE Healthcare®) and sequenced at Myleus Biotechnology Company.

We captured 230 Auchenorrhyncha in both areas using the adhesive traps placed in coconut leaves. Of these, 94% belonged to the families Cixiidae and Derbidae and to the subfamily Deltocephalinae (Cicadellidae), taxa classified as the most important group of phytoplasmas vectors. It is noteworthy that the vector *H. crudus* (Cixiidae) was captured in very high abundances, totaling 87% of the specimens collected. Molecular identification based on COI gene (583 bp and 95% identities) confirmed that samples were *H. crudus* based on GenBank sequences (Genbank Accession Number MK138530). *Haplaxius crudus* were visually observed in the canopy of coconut trees in the Para state, being captured 196 individuals using a buccal aspirator (Fig 2c). Also, the fungi *Fusarium* sp., *Paecilomyces* sp., and *Penicillium* sp. were observed colonizing *H. crudus* in the field.

This is the first record of the occurrence of LY vectors in Brazilian coconut plantations. Interestingly, high populations of *H. crudus* occurred in the Brazilian Green Dwarf Jiqui, but this planthopper was not found in the area of Dwarf vs. Dwarf hybrids. *H. crudus* nymphs develop on roots of grasses or cyperaceae, where they find moisture, shading and feeding, while adults feed on the palm phloem sap of coconut and palm trees (Howard *et al* 1983). In the two Auchenorrhyncha sampling areas, Kudzu *Pueraria* sp. (Fabaceae) was used as cover crop, which has not been reported as a host of *H. crudus*. However, in the Brazilian Green Dwarf Jiqui coconut plantation where *H. crudus* was captured, in addition to this Fabaceae, there was the presence of grasses and a water source. More research is needed to assess the potential hosts of nymphal stages of *H. crudus* in this region aiming at implementing strategies to manage this vector. Furthermore, based on our findings, further studies should determine the role of fungi, especially *Paecilomyces* sp., as biological control agents of *H. crudus*.

Our research highlights the need to carry out bioassays of LY phytoplasma transmission in case the disease enters Brazil. Several studies for phytoplasma transmission in coconut or palm trees were carried out with potential insect vectors. It was successful in two cases: *H crudus* in Florida (Howard *et al* 1983) and *Recilia mica* (Cicadellidae) for blast in Côte d'Ivoire (Desmier De Chenon 1979; Julia 1979). Phytoplasmas of the group 16S rDNA IV were also experimentally transmitted to the palm *Pritchardia pacifica* by *H. crudus* in Mexico, but not on coconut tree (Dzido, Dollet, Julia, Narvaez, and Oropeza not published). However, no successful transmission of LY phytoplasma was obtained in Jamaica with *H. crudus*. In Africa, where *H. crudus* is absent, all attempts to transmit experimentally LYTS in Ghana and Tanzania, failed. Several Auchenorrhyncha insects have been tested without success in Ghana (Philippe *et al* 2009; Pilet *et al* 2009). Positive insects are not necessarily vectors as they might have acquired the pathogens by ingestion or possibly transovarial or sexual transmission.

This is the first report of the *H. crudus* on coconut trees in the Brazil. These findings are of great scientific relevance since *H. crudus* is the proven vector of LY, a major coconut disease that could negatively impact the Brazilian coconut industry and this knowledge could be used in contingency measures in the case of LY entrance in the country.

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