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Arocha, Y., Pinol, B., Lopez, M., Miranda, I., Almeida, R., Wilson, M. and Jones, P. 2007. 'Bunchy top symptom' of papaya in Cuba: new insights. *Bulletin of Insectology*. 60 (2), pp. 393-394.

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## 'Bunchy top symptom' of papaya in Cuba: new insights

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### Abstract

Papaya is an important export crop in Cuba. A disease named Bunchy top symptom (BTS) was first observed in 2003 from Villa Clara province, but is now widespread throughout the country. A nested PCR assay with 16S rDNA phytoplasma primers P1/P7 and R16F2n/R2 was used to index more than 2200 papaya plants, weeds and insect samples collected between November 2005 and June 2006. RFLP patterns for all amplicons were identical with *Hae*III, *Rsa*I and *Alu*I enzymes. No rickettsia-like bacteria were found in any of the samples. Phytoplasma rDNA was amplified from 1449 (89.7%) papaya plants with BTS symptoms. Phytoplasma rDNA was also detected in 331 apparently healthy papayas, and other plant species: *Anoda acerifolia* (57), *Euphorbia heterophylla* (73), *Malvastrum coromandelianum* (41) and *Rynchosia minima* (37) and 60/75 batches of *Empoasca papayae*. Sequences from the phytoplasma in papaya (DQ868531), *A. acerifolia* (DQ286950); *E. heterophylla* (DQ286951); *M. coromandelianum* (DQ286952); *R. minima* (DQ868533) and the leafhopper *E. papayae* (DQ868532) all showed 99% similarity to the phytoplasma associated with polygala phyllody, PPhy, (AY787140) belonging to the 16SrII group, 'Candidatus Phytoplasma aurantifolia'. The discovery of the BTS phytoplasma in weed species and a putative vector *Empoasca papayae* suggests, that these plants and the leafhopper may have role in the spread of this disease.

**Key words:** phytoplasma, papaya, bunchy top, leafhopper, *Empoasca papayae*.

### Introduction

Papaya in Cuba is affected by BTS, a bunchy top symptom disease, which has been associated with phytoplasmas (Arocha *et al.*, 2003; Arocha *et al.*, 2005; Arocha *et al.*, 2006). Some of the symptoms are similar to those described for papaya bunchy top (PBT) associated with a rickettsia (Davis *et al.*, 1998), and exhibit stunting, shortening of internodes, leaf chlorosis, deformation, necrosis, crinkling, and yellowing.

Phytoplasmas have been reported for papaya diseases in Australia (Gibb *et al.*, 1996) and Israel (Gera *et al.*, 2005), however, no vectors have yet been identified.

Little is known about the epidemiology of phytoplasma diseases of papaya, their insect vectors or natural reservoirs. There must be a three-way interaction between phytoplasmas, their plant hosts, and insect vectors involved in an epidemic. The level of disease in a crop will depend on the vector populations and abundance of reservoir hosts in the vicinity.

Recently, a phytoplasma member of 16SrII group was identified in eastern Cuba (Arocha *et al.*, 2006), and in *Empoasca papayae* as a putative leafhopper vector (Arocha *et al.*, 2005).

We report on the impact of BTS phytoplasma (BTSp) associated with the disease and the possible contribution of BTS weed reservoirs and infection levels of the putative leafhopper vector to BTS epidemiology.

### Materials and methods

Leaf samples from 2228 papaya plants with (1616) and without (612) BTS symptoms, and 326 assorted

weed species with and without symptoms of yellowing, little leaf, phyllody, virescence and witches' broom, and 226 adult species of *E. papayae* were collected from papaya plantations in western, central and eastern regions of Cuba. DNA was indexed by nested PCR with phytoplasma 16S rDNA primers P1/P7-R16F2n/R2. DNA from papaya and *E. papayae* were also tested by PCR with primers PBTF1/PBTR1, amplifying the rickettsial succinate dehydrogenase gene (*sdhA*) (Davis *et al.*, 1998). PCR products were digested with *Hae*III, *Rsa*I and *Alu*I. *In silico* restriction-site maps were generated using RESEARCH program (Rothamsted Research, UK). P1/P7 PCR products were sequenced (www.dnaseq.co.uk), and sequences compared with others in Genbank, aligned by Clustal W from MEGA program version 3.1, and phylogeny produced using the neighbour-joining method with *A. laidlawii* as the outgroup. A method of multiple comparison proportions STATISTICA (StatSoft Inc., 1998) was used to calculate the infection index and asymptomatic infection level in the different provinces and regions.

### Results

PCR products with identical *Hae*III RFLP patterns were produced by 1449/1616 papaya plants with and 331/612 without symptoms, 60/75 batches of *E. papayae* and all phytoplasma positive controls. Phytoplasma rDNA was also amplified from 208/326 weed samples: *A. acerifolia* (57/60), *E. heterophylla* (73/74), *M. coromandelianum* (41/44) and *R. minima* (37/38). PCR amplicons from the BTS plants, *E. papayae* and weed samples

gave identical *RsaI* and *AluI* restriction patterns to those of FBP, PWB and SPL, while *AluI* patterns were similar to those of FBP and SPL. DNA sequences from papaya (DQ868531), *A. acerifolia* (DQ286950), *E. heterophylla* (DQ286951), *M. coromandelianum* (DQ286952), *R. minima* (DQ868533) and *E. papayae* (DQ868532) showed a 100% identity to those of the phytoplasma identified in eastern Cuba in 2005 in papaya (DQ286948) and *E. papayae* (DQ286949), that shared a 99% identity to that of PPhy (AY787140), which is from a phytoplasma belonging to the 16SrII group. Comparisons of infection indices showed the highest index of infection for Matanzas, followed by Pinar del Río, then Havana. The highest asymptomatic infection level was for Pinar del Río province.

## Discussion

Association of phytoplasmas with BTS in Cuba was confirmed by detecting phytoplasma DNA in 89.7% of symptomatic papaya plants, and reinforced by the non detection of neither rickettsia nor virus particles. Phylogeny showed BTSp embraced in the same phylogenetic branch that contains phytoplasmas identified in 2005 in papaya and *E. papayae* corresponding to group 16SrII. *A. acerifolia*, *E. heterophylla*, *R. minima* and *M. coromandelianum* were identified as phytoplasma hosts that could have a role in the epidemiology of BTS. Plants without symptoms also tested positive for BTSp, 41 of *M. coromandelianum* and 37 of *R. minima*, thus, the weed population is an important reservoir of inoculum whose control could help reduce the disease incidence in papaya. Further studies will be required to confirm *E. papayae* as the BTSp vector and to identify those factors involved in the development of the disease.

The highest BTS phytoplasma asymptomatic infection level (73.3%) was scored in the western region, however, 54% of the asymptomatic papaya plants collected throughout the country were positive for BTSp. Further studies will be required to understand the genetic mechanisms of BTSp latent infection in the papaya host and the environmental factors involved.

*E. papayae* is reported as the natural vector of the rickettsia, however *E. papayae* collected from the BTS affected papaya plantations in this study were all negative when indexed with the rickettsial primers. These findings along with the fact that *E. papayae* is active on infected papayas, point to *E. papayae* as the potential vector of BTS in Cuba.

Matanzas and the western region scored the highest BTSp infection percentages. Since one of the main papaya germplasm collections is located in Matanzas, and that the western region hosts a high number of private papaya growers, studies on the aetiology, transmission mechanisms, epidemiology and plant-vector-phytoplasma interactions are urgently needed for BTS. This should allow the development of sustainable crop protection strategies for the control of BTS in Cuba.

## Acknowledgements

This research was supported by the International Foundation of Science (IFS), Stockholm, Sweden, through a grant to Y. Arocha Rosete. Work in UK was done under Defra plant health licence no. PHL: 174B/4612 (09/2003). The Global Plant Clinic is supported by the Department for International Development, UK.

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