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RESEARCH ARTICLE

Detection and variability of the lethal yellowing group (16Sr IV) phytoplasmas in the *Cedusa* sp. (Hemiptera: Auchenorrhyncha: Derbidae) in Jamaica

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Cedusa sp.; Derbidae; Hemiptera; lethal yellowing group; phytoplasma; 16S ribosomal RNA.

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Abstract

Lethal yellowing (LY) group phytoplasma was detected in members of the *Cedusa* species of Derbids from Jamaica by nested PCR assay employing the rRNA primer pairs P1/P7 and LY16Sf/LY16Sr. A 1400-bp product was obtained from 13/43 Derbids that were analysed. Restriction fragment length polymorphism analysis of the nested PCR product revealed variations in 6/13 Derbids analysed, suggesting that the planthoppers could be infected with different strains of the LY group phytoplasma. Sequence analysis of the 16S rDNA gene determined that the percentage of similarity of the phytoplasma strains in the Derbids ranged from 97–98% to the phytoplasma strain found in coconuts in Florida (LYFL-C2) and Jamaica (LYJ-C8). The phytoplasma strains in the Derbids clustered together with the Jamaican and Florida coconut LY phytoplasma, as well as other characterised strains composing the LY phytoplasma (16Sr IV) group.

Introduction

Phytoplasmas are nonculturable degenerate gram-positive prokaryotic bacteria with a low G + C content (Kollar & Seemüller, 1989). They lack a cell wall and are closely related to mycoplasmas and spiroplasmas and are classified in the group Mollicutes (Doi *et al.*, 1967; Lim & Sears, 1989; Namba *et al.*, 1993; Gundersen *et al.*, 1994; Seemüller *et al.*, 1994.). Phytoplasmas are phloem limited and are transmitted by phloem-feeding insects of the order Hemiptera (Banttari & Zeyen, 1979; Tsai, 1979). Phytoplasmas have been known to cause over 700 diseases in hundreds of plant species. These include ornamental as well as agricultural crops (McCoy *et al.*, 1989). Phytoplasmas are among the most poorly characterised groups of plant pathogens. This is because of the inability to culture them *in vitro* and isolate them from the relatively small amount of phloem tissue present in plants (Christensen *et al.*, 2004; Weintraub & Beanland, 2006).

The relationship between phytoplasmas and their vectors is not fully understood. Following acquisition, there is a latency period, during which the phytoplasma replicates in the body of the vector (Purcell, 1982; Kirkpatrick, 1992). The phytoplasma is then thought to enter the epithelial cells of the midgut and replicate within a vesicle or pass between two midgut cells and through the basement membrane to enter the haemocoel (Purcell, 1982; Kirkpatrick, 1992). The phytoplasmas circulate in the haemolymph where they replicate. In order for the phytoplasma to be transmitted, it must enter specific cells of the salivary glands and the levels in the posterior acinar cells must accumulate to a high level before it can be transmitted. An insect is classified as a 'dead-end' host if the phytoplasma is not able to pass into the haemolymph.

The planthopper *Myndus crudus* was shown in transmission trials to be a vector of lethal yellowing (LY) in Florida (Howard *et al.*, 1983). LY is a fatal disease of the coconut palm (*Cocos nucifera* L.) and at least 35 other palm species in the Americas (Harrison *et al.*, 1999) and has killed millions of plants in the Caribbean over the past 40 years. Coconut LY (CLY) disease is the single most important disease affecting coconuts in Jamaica and within 6 years, the disease had destroyed all the Malayan Dwarf palms in eastern Jamaica.

No insects so far have been identified in Jamaica to be the carriers/vectors of phytoplasma. In this study, adult planthoppers in the family Derbidae, more specifically the *Cedusa* species were found to be the predominant insects occurring on coconuts in Jamaica. The occurrence of members of the *Cedusa* species in Jamaica is not well documented. Here we report the presence of different strains of the CLY phytoplasma in an unclassified member of the *Cedusa* species in Jamaica. Restriction fragment length polymorphism (RFLP) and sequence analyses of the PCR-amplified rDNA gene showed that it was a member of the phytoplasma LY16Sr IV group (lethal yellowing).

Materials and methods

Insect collection

Insects were collected from LY-infected and uninfected coconut palms from 13 coconut-growing farms islandwide, using a 50-mL Corning tube moistened with ethanol. The insects were stored immediately in 70% ethanol and placed on ice, after which they were stored at −20°C until analysis. Species were identified by M.R. Wilson of the National Museums and Galleries of Wales, Cardiff, UK.

DNA extraction

Total nucleic acid was extracted from individual insects using a modification of the protocol described by Harrison *et al.* (1996). Ethanol was blotted from the insects before the extraction procedure. Individual insects were ground in sterile 1.5-mL microfuge tubes containing 500 µl of DNA extraction buffer [2% cetyltrimethylammonium bromide (CTAB); 100 mM Tris-HCl, pH 8.0; 20 mM disodium ethylenediaminetetra-acetic acid (EDTA), pH 8.0; 1.4 M NaCl; 1% polyvinylpyrollidone (PVP-40) and 1% 2-mercaptoethanol]. Extracts were incubated at 60°C for 30 min, cooled and mixed with 500 µl of chloroform/isoamyl alcohol (24:1 v/v). Mixtures were emulsified by vortexing at high speed and then centrifuged at 16 000 *g* for 15 min. Each upper phase was transferred to a second 1.5-mL microfuge tube and nucleic acids were precipitated by adding 1 volume of cold isopropanol. Nucleic acids were pelleted by centrifugation at 16 000 *g* for 15 min, washed in 70% ethanol and dried briefly in a speed vac. The pellet was resuspended in 20 µl of Tris-EDTA buffer, pH 7.5 and stored at 4°C.

Polymerase chain reaction analysis

Nucleic acid samples extracted from individual insects were first diluted 1:50 with sterile deionized water, and 2 µl of the diluted solution was used as template in each PCR reaction mixture. Phytoplasma 'infection' was investigated initially by PCR employing phytoplasma-universal rRNA primer pair P1 (Deng & Hiruki, 1991) and P7 (Smart *et al.*, 1996). The DNA extracted from healthy coconuts (MayPan) was used as the negative control and the DNA extracted from 'infected' coconut plants (MayPan) as the positive control. Amplifications were performed in 25-µl final volumes each containing 2 µl of sample DNA template, 50 ng of each primer, 200 µM dNTP, 2.5 U of PuReTaq DNA polymerase, 10 mM Tris-HCL, 50 mM KCl and 1.5 mM $MgCl_2$ (Amersham Biosciences, Piscataway, NJ). P1/P7-primed PCR was performed using the following parameters: 3 min at 95°C, followed by 30 s at 94°C, 53°C for 90 s, 72°C for 90 s for 35 cycles and 10 min at 72°C. Products of the P1/P7-primed PCR were diluted 1:10 with sterile deionized water, and 2 µl of each dilution was used as template for reamplification by PCR using the rRNA primers LY16Sf and LY16Sr (Harrison *et al.*, 2002*a*). For nested PCR, the following parameters were used: 3 min at 95°C followed by 30 s at 94°C, 56°C for 60 s, 72°C for 90 s for 35 cycles and 10 min at 72°C. Aliquots (10 µl) of each final reaction mixture were electrophoresed through 1% agarose gels using TAE (40 mM Tris-acetate, 1 mM EDTA) as running buffer. Products in gels were stained with ethidium bromide, visualised by UV transillumination and photographed.

RFLP analyses of PCR products

Nested PCR products amplified from insects as well as from the reference phytoplasma DNA used in this study were analysed by separate digestion with restriction endonucleases *Alu*I, *Hin*fI, *Rsa*I, *Hha*I, (Invitrogen, Carlsbad, CA) and *Msp*I (Promega, Madison, WI) at 37°C and *Taq*I (Invitrogen, Carlsbad, CA) at 60°C for a minimum of 16 h. Digests were electrophoresed through 8% nondenaturing polyacrylamide gels using TBE (90 mM Tris-borate, 2 mM EDTA) as running buffer. The RFLP profiles of digested DNA were visualised and recorded as previously described.

Cloning and sequencing of PCR products

LY16Sf/LY16Sr-primed rDNA nested PCR products were purified separately on spin columns (QIAquick PCR Purification Kit, Qiagen, Valencia, CA) and eluted with sterile deionized water. Each purified product was then cloned in vector pGEM-T (Promega, Madison, WI) and *Escherichia coli* XL1 Blue cells (Stratagene, La Jolla, CA) according to manufacturer's instructions. Recombinant plasmid DNA was extracted using the Qiagen Plasmid Minikit (Qiagen,

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Valencia, CA). Representative rDNA gene clones were sequenced at the Center for Comparative Functional Genomics, University of Albany, NY.

Nucleotide sequence and phylogenetic analysis

DNA sequences were edited using EditSeq option of (DNASTAR) program (DNASTAR, Inc., Madison, WI). The 16S rDNA gene sequence similarities between strains were determined after alignments were generated using the MegAlign option of DNASTAR. For phylogenetic analysis, 16S rDNA gene sequences of the Jamaican LY strain as well as 18 other phytoplasmas representing 14 primary phytoplasma species (IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group, 2004) and *Acholeplasma laidlawii* (Table 1) were aligned using ClustalX version 1.63b (Thompson *et al.*, 1997). Cladistic analyses were performed with PAUP* version 4.0 (Swofford, 1998). A phylogenetic tree was constructed using the Neighbour-Joining method and the tree was viewed using TreeView (Page, 1996). The phylogenetic tree was constructed using a heuristic search, implementing the tree bisection and reconnection branch swapping algorithm. The number of bootstrap replicates was 2000. Bootstrapping was performed to estimate the stability and support for inferred

Table 1 Description of phytoplasma 16S rDNA gene sequences used in this study

Primary Phytoplasma Species Name	Phytoplasma Strain or Associated Disease	16S rRNA Subgroups	GenBank Accession no.	Reference
Candidatus Phytoplasma asteris	Michigan aster yellows (MiAY)	16SrI-B	M30790	Lee *et al.* (2004)
Candidatus Phytoplasma aurantifolia[a]	Papaya yellow crinkle (PpYC)	16SrII-E	Y100097	IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group (2004)
	Peanut witches' broom (PnWB)	16SrII-A	L33765	Gundersen *et al.* (1994)
Candidatus Phytoplasma aurantifolia	Witches'-broom disease of lime (WBDL)	16SrII-B	UI5442	Zreik *et al.* (1995)
Candidatus phytoplasma cynodontis	Bermuda grass white leaf (BGWL)	16SrXIV	Y16388	Marcone *et al.* (2004)
Candidatus Phytoplasma fraxini	Ash yellows (AshY1)	16SrVII-A	AF092209	Griffiths *et al.* (1999)
Candidatus Phytoplasma luffae	Loofah witches'-broom (LfWB)	16SrVIII-A	L33764	IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group (2004)
Candidatus Phytoplasma japonicum	Japanese hydrangea phyllody (JHP)	16SrI-D	AB010425	Sawayanagi *et al.* (1999)
Candidatus Phytoplasma oryzae	Rice yellow dwarf (RYD)	16SrXI-A	D12581	Jung *et al.* (2003)
Candidatus Phytoplasma palmae	Carludovica palmate yellows (CPY)	16SrIV-D	AF237615	IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group (2004)
	Coconut lethal yellowing, Florida (LYFL-C2)	16SrIV-A	AF498309	Harrison *et al.* (2002*b*)
	Coconut lethal yellowing, Florida (LYFL-C5)	16SrIV-A	AF498308	Harrison *et al.* (2002*b*)
	Coconut lethal yellowing, Florida (LYFL-C8)	16SrIV-A	AF498307	Harrison *et al.* (2002*b*)
	Phytoplasma Cedusa sp. DJ2	16SrIV	DQ286705	This article
	Phytoplasma Cedusa sp. DJ4	16SrIV	DQ286702	This article
	Phytoplasma Cedusa sp. DJ5	16SrIV	DQ286707	This article
	Phytoplasma Cedusa sp. DJ7	16SrIV	DQ286706	This article
	Phytoplasma Cedusa sp. DJ8	16SrIV	DQ286701	This article
	Texas Phoenix decline (TPD)	16SrIV-D	AF434989	Harrison *et al.* (2002*a*)
	Yucatan coconut lethal decline (LDY)	16SrIV-B	Y18753	Tymon *et al.* (1998)
Candidatus Phytoplasma pruni	Clover yellow edge (CYE-C)	16SrIII-B	L33766	IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group (2004)
	Western X (WX)	16SrIII-A	L04682	Seemüller *et al.* (1998)
Candidatus Phytoplasma trifolii	Clover proliferation	16SrVI-A	L33761	Hiruki & Wang (2004)
	Acholeplasma laidlawii		M23932	Weisburg *et al.* (1989)

[a]This phytoplasma was also designated *Candidatus* Phytoplasma australasia but this name has not been retained (IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group, 2004).

clades. The tree was rooted using *A. laidlawii* as the outgroup.

Results

Phytoplasma detection by PCR

A total of 43 Derbids from 13 major coconut-growing locations in Jamaica were assayed individually for the presence of phytoplasma. Phytoplasmal DNA was detected in 13 of the 43 (30%) insects assayed. Reamplification of the P1/P7 products employing the nested rRNA primer pair LY16Sf/LY16Sr yielded a 1.4-kb product (Fig. 1). A 1.4-kb PCR product was also amplified from the CLY phytoplasma reference DNA.

RFLP analyses of phytoplasma 16S rDNA gene sequences

RFLP analyses of the phytoplasma 16S rDNA gene from the insects and the reference samples were carried out using nested PCR products with primers LY16Sr/LY16Sf. Of the 13 insects that tested positive for phytoplasmal DNA, only six showed variations in their banding patterns when analysed using the six restriction enzymes. These are discussed below.

Partial 16S rDNA of the phytoplasma isolates from the insects analysed produced RFLP patterns that were identical to that of the reference strain when digested with the enzyme *Hha*I (Fig. 2A). Analysis using the restriction enzyme *Msp*I produced a profile, which, with the exception of DJ2, was identical to that of the reference strain. This particular phytoplasma strain (DJ2) produced a four-fragment banding pattern instead of three fragments (Fig. 2B). In the sample DJ2, the band at 500 bp was absent and instead a double band could be seen at approximately 300 bp and 350 bp; an additional band was also seen at approximately 120 bp.

Digestion of phytoplasmal rDNA gene using the restriction enzyme *Taq*I produced a RFLP profile that was identical to that of the reference strain in all but three of the phytoplasma strains analysed. These included the phytoplasma strain obtained from the insects DJ5, DJ4 and DJ3. In the case of DJ5, only two bands were common to that of the reference strain (Fig. 2C). These were the bands at approximately 850 bp and 360 bp. The difference in banding pattern could be seen in the band at 650 bp. The band at approximately 150 bp was also absent (Fig. 2C). In the case of DJ3 and DJ54, the band at 850 bp was absent and instead a band at 650 bp could be seen. They, however, shared bands at 360 bp as well as 150 bp with that of the reference strain.

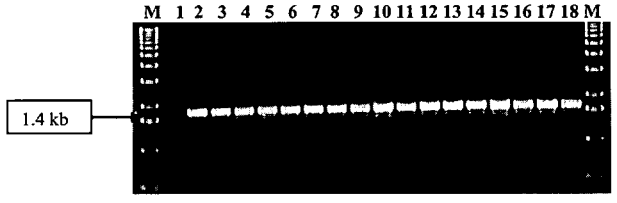

Figure 1 Agarose gel electrophoresis of DNA products amplified by PCR assays from lethal yellowing (LY)-infected coconut palm and members of the *Cedusa* species from Jamaica. DNA products (1.4 kb) were generated by a nested PCR assay using phytoplasma universal rRNA primer pair P1/P7 followed by reamplification of products with rRNA primer pair LY16Sf/LY16Sr. M, 1-kb marker (Invitrogen), lane 1, healthy coconut palm; lane 2, LY-infected coconut palm. Lanes 3–18 insect samples.

Digestion with the enzyme *Rsa*I produced a restriction profile that was identical to that of the reference strain with the exception of DJ6. In analysing the restriction profile of DJ6, the band at 500 bp was absent and an additional band could be seen at 100 bp (Fig. 2D, lane 7). Analysis of the restriction profile produced with the enzyme *Hin*fI showed the presence of three different restriction banding patterns. The first was a three-fragment banding pattern that was similar to that of the reference strain (Fig. 2E, lanes 1–3, 6 and 8). A second profile was observed in insect samples, DJ3 and DJ4; in this profile, a three-fragment banding pattern was seen with the band at 370 bp being absent and instead a band at approximately 179 bp could be seen (Fig. 2E, lanes 4 and 5). The third banding pattern was observed in samples DJ6 and DJ8; in this instance, a four-fragment profile was observed (Fig. 2E, lanes 7 and 9). They shared only two bands in common with the reference strain; these were 1000 bp and 100 bp. Additional bands could be seen at 222 bp and 150 bp. Two different restriction profiles were observed in the *Alu*I digestion of the phytoplasma rDNA gene. With the exception of DJ3 and DJ4, all of the phytoplasmal rDNA gene analysed produced a profile that was identical to that of the reference strain. In both the cases, the band at approximately 120 bp was missing and instead a band could be seen at approximately 90 bp (Fig. 2F, lanes 5 and 6).

In summary, these results suggest a total of six different LY group phytoplasma strains detected among the 13 insects analysed. These were found in the samples DJ2, DJ3, DJ4, DJ5, DJ6 and DJ8. With the exception of the enzyme *Hha*I, all of the restriction enzymes used were able to differentiate at least one strain. However, most of the variation was seen in the profile generated using *Hin*fI and *Taq*I as the restriction enzyme.

Phylogenetic analysis

The nucleotide sequences of the phytoplasma strain found in the *Cedusa* sp. of planthoppers were deposited in

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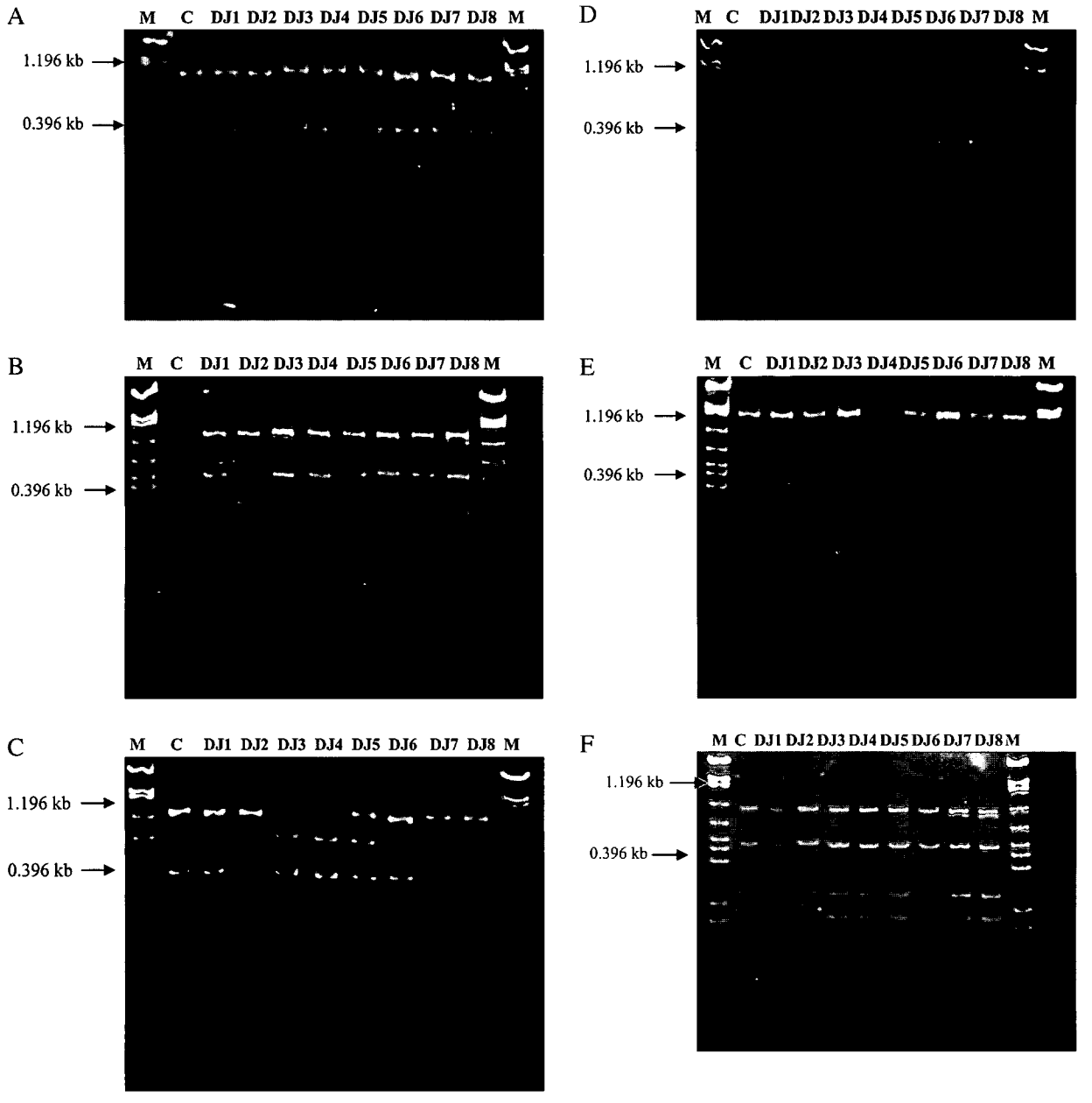

Figure 2 Comparison of RFLP patterns of rDNA products (1.4 kb) of lethal yellowing (LY) phytoplasmas amplified by nested PCR assay employing rRNA primers P1/P7 and LY16Sf/LY16Sr. DNA products were digested with restriction endonucleases and separated by electrophoresis through 8% polyacrylamide gel. (A) *Hha*I, (B) *Msp*I, (C) *Taq*I, (D) *Rsa*I, (E) *Hin*fI, (F) *Alu*I. M, pGEM DNA marker (Promega); C, LY-infected coconut palm; DJ1–DJ8, insect samples *Cedusa* sp. The size of the pGEM molecular marker is as follows: 126, 179, 222, 3550, 396, 460, 517, 676, 1196, 1605, 2645 base pairs.

the GenBank under accession numbers DQ286701, DQ286702, DQ286705, DQ286706 and DQ286707. Pairwise comparisons between the 16S rRNA gene sequences of the LY phytoplasma strain in the Jamaican MayPan coconut variety and the phytoplasma found in insects had nucleotide identities ranging from 97–98%. A similar result was obtained when the 16S rRNA gene sequences of the phytoplasma found in the insects analysed were compared with that of the phytoplasma strain found in Florida (LYFL-C2) (Harrison *et al.*, 2002*b*). The levels of sequence similarities of 16S rDNA gene sequences indicate that the LY group phytoplasma strain found in these insects are similar to the LY phytoplasma strain in coconuts from Jamaica and Florida.

A phylogenetic distance tree was constructed using 16S rRNA gene sequences isolated from the insects collected in this study and comparable sequences of 18 additional strains representing 14 previously established phytoplasma groups (Fig. 3). Tree branching orders resolved by the analysis were similar to and supported the same phylogenetic groups identified in other recent studies (Harrison *et al.*, 2002*a*,*b*). Tree branching patterns verified that the phytoplasma strain in the insects analysed was a part of the existing lineage of subgroup 16Sr IV strains.

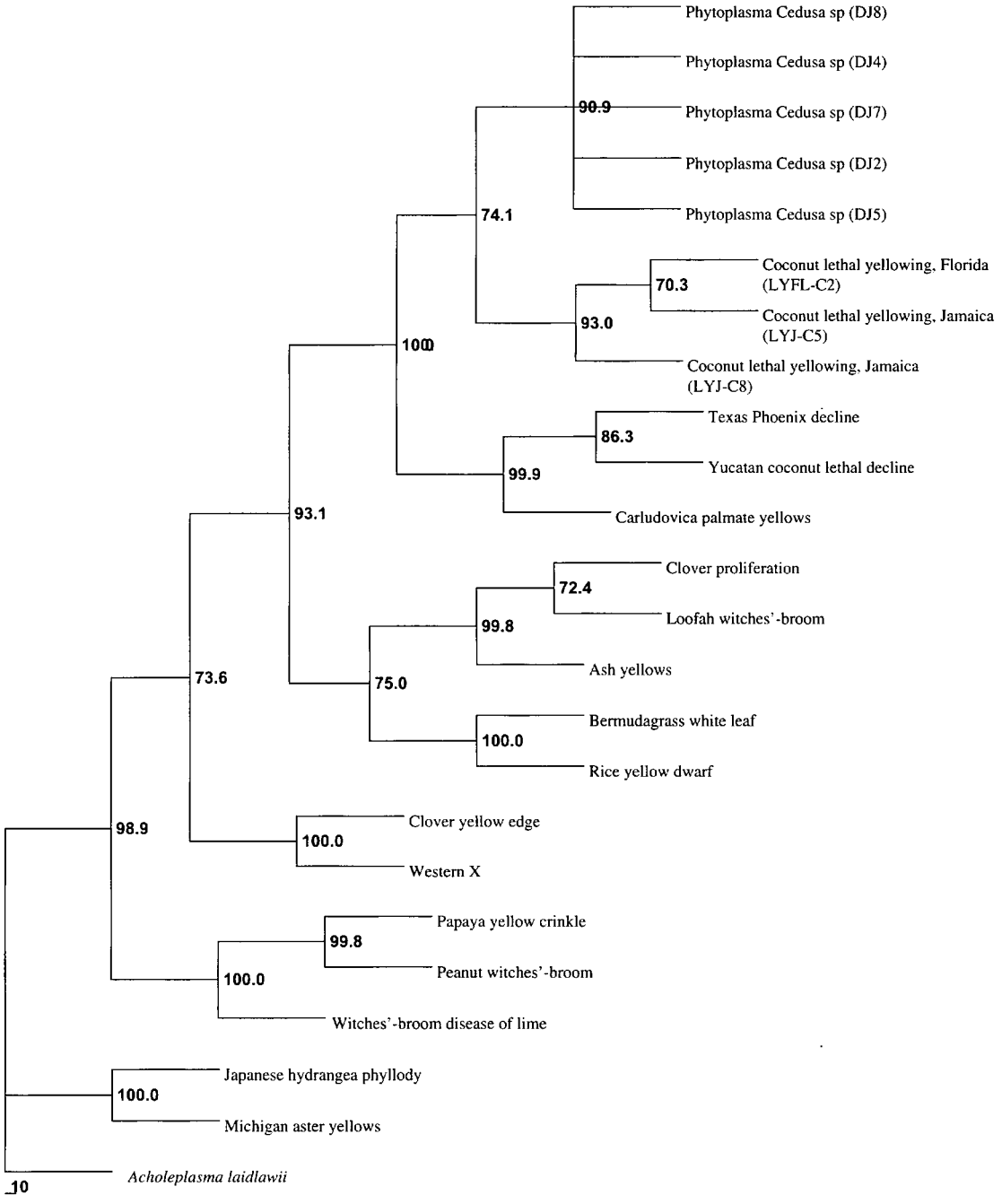

Figure 3 Phylogenetic tree constructed by the Neighbour-Joining method of 16S rRNA gene sequences from 23 phytoplasmas and *Acholeplasma laidlawii*, with *A. laidlawii* as the outgroup. The phylogenetic tree was constructed by using a heuristic search, implementing the tree bisection and reconnection branch swapping algorithm to find optimal trees. The number of bootstrap replicates was 2000. Bar length represents inferred character-state changes.

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Discussion

Reliable detection of phytoplasmas in insect vectors has been reported both by the use of DNA probes (Nakashima *et al.*, 1993) and PCR (Vega *et al.*, 1993; Harrison *et al.*, 1995). In this study, specific primers were used to screen potential insect vectors for the presence of the CLY phytoplasma. PCR products were amplified from 13 of the 43 insects screened. All of the insects screened were planthoppers belonging to the family Derbidae and were of the *Cedusa* sp. These insects have yet to be fully identified as this particular species has not been classified before.

The proportion of insects in which phytoplasmas were detected was 30% of those assayed. This proportion is inconsistent with findings from other phytoplasmal systems. Mpunami *et al.* (1996, 2000) in their search for vectors of coconut lethal disease in Tanzania were able to amplify phytoplasma rDNA from only 7 of 5000 individual insects tested. The higher detection rate of the phytoplasma in the insects tested in this study could be attributed to the nested PCR analysis, which has enabled extremely sensitive detection of phytoplasmas in both plants and insects (Danielli *et al.*, 1996; Gunderson & Lee, 1996). Of the 13 insects that were carrying the LY group phytoplasma, 6 were found to be carrying a different strain of the LY group phytoplasma. The six different LY group phytoplasma strains could be differentiated by RFLP analysis using endonucleases *Alu*I, *Hin*fI, *Rsa*I, *Taq*I and *Msp*I. Both insects and plants are natural hosts of phytoplasmas (Lee *et al.*, 1998). The host range in insect vectors and plants vary with phytoplasma strains. Some phytoplasmas have a low insect vector specificity whereas others have a very high vector specificity (Lee *et al.*, 1998). The LY group phytoplasma can be classified as having a very high insect vector specificity as the only vector that has been identified to date is *Myndus crudus* (Howard *et al.*, 1983). The present findings in this study have serious implications for LY epidemiology studies. The fact that a new member of the *Cedusa* sp. has been discovered, coupled with the fact that they were carrying different strains of the LY group phytoplasma, raises the possibility that they could be a vector of phytoplasmas associated with CLY disease. It may also explain the recent spread in the CLY disease in Jamaica. Before the onset of the epidemic, there was no mention of this particular species of *Cedusa* in Jamaica; however, in recent times, they can be found in almost every farm in Jamaica. This was not observed with *M. crudus* irrespective of the time of day when sampling was performed. In addition to the LY phytoplasma strain, the insects were also capable of acquiring several different strains of this phytoplasma. It is known that many vectors can transmit more than one type of phytoplasma (Lee *et al.*, 1998); however, transmission studies will have to be carried out to determine whether this particular member of the *Cedusa* species is capable of actually transmitting these different strains of the LY phytoplasma. There are more than 1000 species of Derbids worldwide. However, none has ever been reported as transmitters of plant pathogens. This is the first time that CLY phytoplasma is being reported in Derbids in Jamaica.

Pairwise comparisons showed that the percentage of similarity of the phytoplasmas in the Derbids analysed ranged from 97–98% to the LY phytoplasma from Jamaica and Florida, which would indicate that they are a part of the 16Sr IV group. This is coupled with the fact that the phytoplasma was amplified using 16S rRNA primers that were specific for the LY group (16Sr IV) phytoplasmas. According to the IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group (2004), a sequence similarity of less than 97.5% can be classified as being a new species of phytoplasma; hence, the possibility exists that a new species of LY phytoplasma is present in Jamaica. However, more analysis will have to be carried out before a definitive statement can be made. The phytoplasma obtained from the *Cedusa* species, all clustered together, forms what appears to be a new subgroup within the major LY phytoplasma group (16Sr IV) (Fig. 3). Current phytoplasma classification schemes provide recognition of subgroups within major phytoplasma groups (Lee *et al.*, 1993; Gundersen *et al.*, 1996); within the LY phytoplasma group three subgroups already exist (Table 1). It has been theorised that these subgroups could have arisen through evolution from an ancestral population by inhabiting different plants and insect hosts (Davis & Sinclair, 1998).

Unfortunately with 16S sequences, variability in phytoplasma strains is very difficult to detect. This can be attributed to the conserved nature of these sequences, hence the need for more sensitive RFLP analysis. The RFLP analysis detected numerous variations in the rDNA of the phytoplasma strain in the insects (Fig. 2). The enzymes *Taq*I and *Hin*fI allowed the differentiation of three different strains of CLY phytoplasma (Figs 2C and 2E). Because of overlapping host plants of many insect vectors and their capability to transmit more than one phytoplasma, it is not surprising that many of these vectors are able to carry dual or multiple phytoplasmas.

In a previous study conducted (S.E. Brown, B.O. Been, K. Johnson & W.A. McLaughlin, unpublished data) on weeds in coconut-growing areas, it was noted that the

16S rRNA genes amplified from the insect DJ2 and from the weed *Macroptilium lathyroides* produced the same RFLP banding pattern when digested with the restriction enzyme *Msp*I (Fig. 2B). This would imply that they are probably the same strain. The possibility exists that this insect could be a vector of this particular strain of LY group phytoplasma in *M. lathyroides*. This opens the door to the question of ecological niches of phytoplasmas. Isolation of a phytoplasma within a particular plant host may largely reflect insect vector feeding habits or plant host susceptibility to that phytoplasma (Lee *et al.*, 1998).

The LY disease remains a serious threat to coconut cultivation in Jamaica; however, little is known about the ability of insect vectors to carry multiple phytoplasmas and transmit them. This information is necessary for interpreting any proposed mechanism for the emergence of new LY phytoplasma strains. Little information is known about how various phytoplasmas become associated with plants and insect vectors. Each disease is the consequence of vector–phytoplasma–plant interactions. Better understanding of phytoplasma ecology is pertinent for the development of efficient control measures to combat disease. Some emphasis has been placed on phytoplasma–plant relationship; however, information concerning vector–phytoplasma and vector–plant relationships or interactions is very limited. This information is necessary for understanding most phytoplasma-associated disease including the LY disease in Jamaica. It is hoped that the presence of LY group phytoplasma in Derbids will serve to assist in the understanding of the recent outbreak of the LY disease in Jamaica as it lends credence to the theory that a different vector may be involved in the spread of this disease and that the LY phytoplasma may have evolved over time.

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