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First report of a phytoplasma associated with abnormal proliferation of cladodes in cactus pear (*Opuntia ficus-indica*) in Italy

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While Mexico is the main cactus pear-producing country, Italy is the most important producer in the Mediterranean basin. No phytoplasma disease of cactus pear has been reported, despite previous detection of phytoplasmas in related species such as *Opuntia tuna* (Casper *et al*., 1970) and *Opuntia linguiformis* showing witches' broom symptoms (Cai *et al*., 2002). In 2003, three cactus pear plants showing abnormal growth were observed in the DISTEF collection. The plants showed severe proliferation of cladodes with lack of flowers, fruits and spine production. Viral particles were not observed by transmission electron microscopy in sap from any of the affected plants.

Total DNA was extracted from the affected plants and from two symptomless cactus pears as described by Cai *et al*. (2002). This was used as template for phytoplasma-specific 16S rDNA PCR amplification using one of three universal primer pairs: P1/P7, R16f2/r2 (Lee *et al*., 2000) or fU5/rU3 (Lorenz *et al*., 1995). DNA preparations from phytoplasma reference strains maintained in periwinkle were used as positive controls. To produce enough amplicon for further characterization by RFLP analysis, nested primer pair R16f2/r2 was used to reamplify P1/P7-primed rDNA products. RFLP analysis was performed with restriction enzymes *Alu*I, *Hha*I, *Hpa*II, *Mse*I and *Taq*I.

Phytoplasma-specific PCR products were amplified from all three plants showing symptoms with P1/P7 and fU5/rU3 primers by direct PCR, and with R16f2/r2 primers by nested PCR, but symptomless plants were always negative in all three PCR assays. The RFLP patterns obtained from analysis of rDNA amplicons from the former samples were identical to the pattern obtained for reference strain faba bean phyllody phytoplasma, a member of the 16S rDNA RFLP subgroup 16SrII-C. This is the first report of a phytoplasma infecting *O. ficus-indica*.

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First report of a lethal yellowing phytoplasma in *Thrinax radiata* and *Coccothrinax readii* palms in the Yucatan Peninsula of Mexico

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Lethal yellowing (LY) disease associated with phytoplasmas of the 16Sr IV group (now reclassified as '*Candidatus* Phytoplasma palmae') has been present in the Yucatan Peninsula of Mexico for more than 25 years, and has killed most of the Atlantic Tall coconut palms. Many other native palm species such as *Thrinax radiata* and *Coccothrinax readii* show no symptoms and were not considered to be susceptible. To determine if these palms are immune or tolerant to infection by the LY phytoplasma, trunk wood samples were collected from 10 apparently healthy palms of each species from the Yucatan coastal location of Chicxulub Puerto. Total DNA extracted from each sample was tested for phytoplasma by nested PCR, using phytoplasma universal rRNA operon primer pair P1/P7, followed by LY group-specific 16S rRNA gene primer pair LY503f/LY16Sr (Harrison *et al*., 1999). Phytoplasma-specific bands were amplified from five *T. radiata* and eight *C. readii* palms and LY-positive controls, but not from DNA from palms of both species previously identified as LY-free.

RFLP profiles obtained by digesting nested PCR products with *Alu*I endonuclease were identical for all phytoplasma-positive palms, and to that of the Florida strain of the LY phytoplasma (Harrison *et al*., 1999). The patterns differed from the RFLP profiles of Mexican Pacific coconut leaf yellowing strains (Harrison *et al*., 2002). The phytoplasma 16S rDNA sequences of PCR amplicons from *T. radiata* and *C. readii* (GenBank accessions AY919862 and AY919863) were identical to each other and to that of the LY phytoplasma (GenBank accession AF498309). The occurrence of LY phytoplasmas in these newly identified, albeit symptomless, palm hosts suggests that they could act as a permanent source of inoculum that would contribute to the epidemic in Yucatan.

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