New Disease Reports

First report of molecular identification of '*Candidatus* Phytoplasma pyri' in pear trees in Belgium

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Pear decline phytoplasma (PD) is a quarantine pest ('harmful organism') for the European Union that has been assigned to the *Candidatus* taxon, '*Candidatus* Phytoplasma pyri'. In October 2010, 137 samples consisting of leaves or roots from symptom-bearing pear trees showing early yellow or red discoloration (Fig. 1) were collected in pear production orchards in northeast Belgium.

Total DNA was extracted from 0.5 g of leaf midribs using a simplified CTAB extraction method (ANSES, 2010). Total DNA was used as a template for phytoplasma testing in a direct PCR assay using the universal primer pair fU5/rU3 (Lorenz *et al*., 1995) that targets the phytoplasma 16S rRNA gene. Amplified 16S rDNA fragments of expected size (approximately 880 bp) were directly sequenced and each obtained sequence was compared to those of reference phytoplasmas in GenBank by BLAST. In order to further study the genetic diversity of the phytoplasma isolates, some positive samples were additionally amplified and directly sequenced using the fHflB3_1/rHflB3 primer pair (Seemüller *et al*., 2011) that targets the putative transmembrane metalloprotease gene (HflB) for phytoplasmas.

Out of the 137 samples, approximately 15% gave positive results for '*Ca*. Phytoplasma pyri' through PCR and sequencing. BLAST analysis of the consensus fU5/rU3 sequence (HF547271) revealed a 100% of sequence identity with that of '*Ca.* Phytoplasma pyri' isolates (16SrX group, subgroup C). Four HflB amplicons of expected size (approximately 530 bp) from samples of four different municipalities were directly sequenced. The obtained sequences were 100% identical in the available regions, and the consensus HflB sequence (HE984352) showed 98.3% sequence identity with that of '*Ca*. Phytoplasma pyri' partial HflB gene, strain PD1 (GenBank Accession No. FM201271). To our knowledge this is the first report of the molecular identification of '*Ca*. Phytoplasma pyri' in Belgian pear orchards.

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Figure 1

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