



Identification of '*Candidatus Phytoplasma fragariae*' (16Sr XII-E) infecting *Corylus avellana* (hazel) in the United Kingdom

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In October 2014 a stand of coppiced *Corylus avellana* (hazel) growing in Surrey (UK) was found exhibiting symptoms of yellowing of leaves, dieback around the leaf margin, a lack of density of the tree canopy and proliferation of small thin branches (less than 0.5 cm diameter), with some trees having died. The plants are believed to have been planted approximately 10 to 15 years ago, and were growing in a clay cap over chalk.

In October 2014 leaf material was collected from one randomly selected tree. DNA was extracted from 0.3 g leaf material using the CTAB method of Doyle & Doyle (1990) and tested by real-time PCR using the universal phytoplasma assay (Hodgetts *et al.*, 2009) where it was found to be positive. Nested PCR of the 16S rRNA gene was performed using universal phytoplasma primers P1 and P7, followed by R16F2n and R16R2 as described in Hodgetts *et al.* (2007), where the sample produced the specific 1,250 bp amplicon. The PCR amplicon was cloned into the pGEM®-T Easy Vector System (Promega, USA) and three randomly selected clones were sequenced. Analysis of the clones revealed the presence of single nucleotide polymorphisms (SNPs) within the sample, containing 1, 2 or 4 SNPs. The most prevalent sequence was submitted to NCBI (Accession No. KP407881). Phylogenetic analysis was undertaken which identified the phytoplasma as a '*Candidatus Phytoplasma fragariae*'-related strain (16SrXII-E) with 99.84 to 99.75 % identity to numerous strains of '*Ca. P. fragariae*', and 99.75 % sequence similarity to the type strain StrawY (DQ086423) (Fig. 1). Additional phylogenetic analysis with representative 16Sr XII phytoplasmas was undertaken (Fig. 2) confirming the placement within '*Candidatus Phytoplasma fragariae*'. In December the original tree was re-sampled, along with seven additional trees in the immediate surrounding area with varying degrees of symptom expression. DNA was extracted from leaf, bud, twig or catkin material as described above and tested by real-time PCR. All eight trees tested positive, and phytoplasmas could be detected in all the tissue types indicating systemic infection of the trees.

'*Ca. P. fragariae*' has been previously found in *Fragaria x ananassa* [strawberry] (Valiunas *et al.*, 2006), *Cordylone* (Hodgetts *et al.*, 2008) and *Sambucus nigra* [elder] (Filippin *et al.*, 2008), whilst hazel has been found infected with '*Ca. P. asteris*', '*Ca. P. mali*', '*Ca. P. pyri*', '*Ca. P. prunorum*' and the peach-X disease group phytoplasma (16SrIII-B). To our knowledge,

this is the first finding of '*Ca. P. fragariae*' in *C. avellana*. Whilst '*Ca. P. fragariae*' was previously identified in a *Cordylone* specimen from Jersey (the Channel Islands) this plant was destroyed and therefore to our knowledge this is also the first finding of '*Ca. P. fragariae*' within the mainland UK. All infected trees will be destroyed, and further surveillance action will be carried out during 2015.

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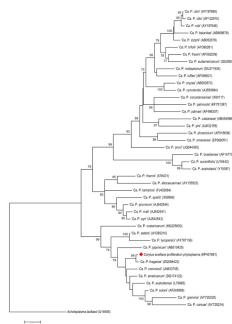


Figure 1

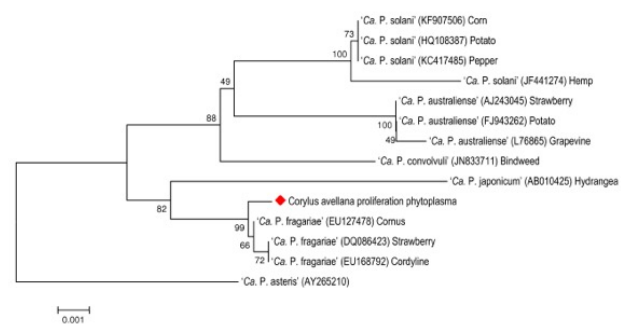


Figure 2

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