New Disease Reports

First finding of *Cryphonectria parasitica* causing chestnut blight on *Castanea sativa* trees in England

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Cryphonectria parasitica has moved north into many European countries since it was first seen in Italy in the 1930's (Biraghi, 1950; Robin & Heiniger, 2001). In November 2011, approximately 90 dead or cankered *Castanea sativa* (sweet chestnut) trees were observed on a farm in Warwickshire, England. The trees had come from a French nursery in 2007 and been planted, together with *Juglans nigra* (walnut) and *Corylus avellanus* (hazel) on a 1.63 ha site. Since then many of the sweet chestnut had not grown well and some had died. Subsequent replanting took place in 2010 with planting stock originating from the same French nursery. Diseased trees exhibited crown dieback, sunken cankers, cracked bark above the root collar and in some cases orange coloured fungal sporulation was visible on the bark (Fig. 1).

Samples were collected from five affected trees and symptom-bearing bark pieces then placed in moist chambers at 18°C for up to 10 days to induce fungal sporulation. Cultures were made from spore masses extruding from the cankered bark onto potato dextrose agar (PDA). Isolations were also made onto PDA from the edge of necrotic lesions visible in the phloem tissue of cankered bark tissue. Fungal cultures were obtained from four of the five trees and the causal fungus was identified through morphological characteristics and DNA sequence data. DNA was extracted using the CTAB extraction protocol (Möller *et al*., 1992) and a portion of the ITS rDNA operon amplified and sequenced following the method of Gryzenhout *et al*., (2004). Resulting sequences were blasted against the nucleotide sequence database of the National Center for Biotechnology Information (NCBI) and closely allied sequences downloaded from NCBI and used to generate a DNA sequence phylogeny (Fig. 2). The DNA sequences of isolates collected in this study were very similar to many *C. parasitica* sequences found on GenBank including AY141859 (99% coverage, 99% identity) and JN942325 (100% coverage, 99% identity). This identification was corroborated by morphological characteristics of the isolated fungus which were the same as those described for *Cryphonectria parasitica* (Sivanesan & Holiday, 1981). Ascospores and asci of the *C. parasitica* isolates collected in England measured (7.5-)8-10(-12) x (3-)4(-5) µm and (32-)38-45(-48) x (5-)6-8(-9) µm respectively, which are in the range known for this species (Sivanesan & Holliday, 1981) (Fig. 3).

Pathogenicity of *C. parasitica* was confirmed through Koch's postulates. Mycelial plugs from three seven-day-old *C. parasitica* cultures were used to inoculate detached twigs of *C. sativa*, while controls used sterile PDA. Each inoculation was carried out in triplicate and inoculated twigs were incubated at 20°C for 5 weeks in a 9h/15 h light/dark cycle following which lesions were measured (Fig. 4). *Cryphonectria parasitica* was re-isolated from lesions confirming the pathogenicity of these isolates. This finding represents an example of incursion by an exotic pathogen into the UK and highlights the importance of plant health inspections and the difficulty of detecting infected plant material at the time of importation. Strategies are underway to manage and contain this outbreak while surveys are being conducted to determine if other sites have infected trees.

Acknowledgements

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Figure 1

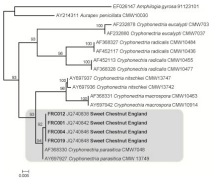

Figure 2

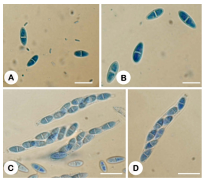

Figure 3

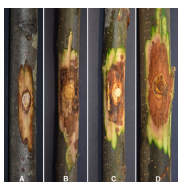

Figure 4

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