First report of *Cryphonectria parasitica* causing Chestnut blight on *Castanea sativa* in the United Kingdom

Gavin C. Hunter[1] (gavin.hunter@forestry.gsi.gov.uk), Barnaby Wilder[2], Ben Jones[2], and Joan Webber[1]
[1]Forest Research, Alice Holt Lodge, Farnham, GU10 4LH, Surrey, U.K.; [2]Forest Services Plant Health Team, Forestry Commission, Bristol, BS16 1EJ, U.K.;

Introduction

- *Cryphonectria parasitica* has caused the deaths of millions of trees in North America and has progressively moved into many European countries since the 1930's (Biraghi 1950, Robin & Heiniger 2001).
- In October 2011, 92 dead and dying *Castanea sativa* (Sweet Chestnut) trees were identified on a farm in Warwickshire, England.
- Affected trees exhibited several symptoms including crown die-back, sunken cankers, cracked and splitting bark above the root collar (Figure 1).
- These trees had been sourced from a French nursery in 2007 (with their plant passports) and planted on a 1.63 hectare site together with Walnut (*Juglans nigra*) and Hazel (*Corylus avellanus*).
- Many Chestnut trees began dying and replanting took place in 2010 with stock from the same French nursery. However, tree growth remained poor and tree mortality continued.
- This study was therefore undertaken to identify the organism causing the observed symptoms and death of Sweet Chestnut.

Figure 1:
Symptoms of Chestnut blight in England.
(A) and (B) Basal cracking of the stem above the root collar.
(C) Stem cracking and a sunken canker.
(D) Orange stromata of *Cryphonectria parasitica*.

Materials and Methods

- Symptomatic bark pieces were incubated in moisture chambers at 18ºC to promote fungal sporulation. Isolations were also made onto PDA from the leading edges of necrotic stem lesions.
- Spore masses extruding from perithecial necks emanating from bark pieces were transferred to Potato Dextrose Agar (PDA).
- Fungal cultures were grown and DNA extracted from these cultures using the CTAB extraction protocol (Möller *et al.* 1992).
- The ITS rDNA operon was amplified and sequenced following the method of Gryzenhout *et al.*, (2004).
- DNA sequences were blasted against GenBank and closely allied sequences downloaded from NCBI and used to generate a DNA sequence phylogeny.
- To assess possible presence of wild-type and hypovirulent forms, a growth study was undertaken. Here all isolates were plated on PDA and incubated at 15, 20, 25 and 30ºC and grown for seven days. Colony diameter measurements were made every day and fungal growth rate determined.

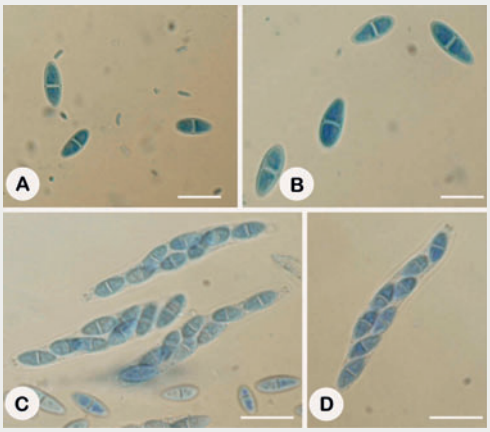


Figure 2:
Morphological characteristics of *Cryphonectria parasitica* collected during this study.
(A) and (B) 1-septate, ellipsoidal ascospore with rounded apices.
(C) and (D) Unitunicate, 8-spored, thin-walled asci.
Scale bar = 10 µm.

Results and Discussions

- Blast results of ITS rDNA sequences and phylogenetic analysis of isolates collected in the UK confirmed their identity as *Cryphonectria parasitica* (Figure 3).
- Ascospores and asci of the *C. parasitica* isolates collected in England measured (7.5-)8-10(-12) × (3-)4(-5) µm and (32-)38-45(-48) (5-)6-8(-9) m respectively, which are in the range known for this species (Sivanesan & Holiday 1981) (Figure 2).
- A total of 77 isolates of *C. parasitica* were collected during this study. Initial growth studies of representatives of these isolates indicated the optimal growth temperature to be 30°C. Variation in growth rate and was observed indicating the possible presence of wild-type and hypovirulent forms in the population (Figure 4).

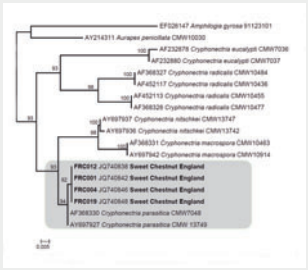
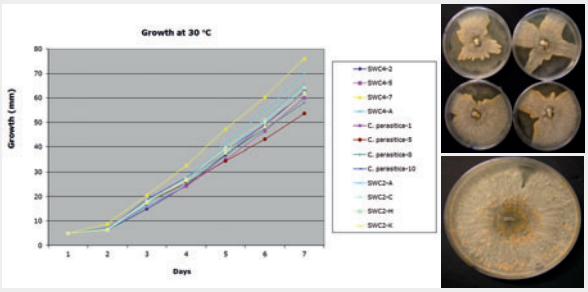


Figure 3 (left):
Maximum Likelihood phylogram of ITS rDNA sequences representing *Cryphonectria* species obtained in RAxML (Stamatakis *et al.*, 2008). Isolates in bold represent *C. parasitica* isolates collected during this study with their associated GenBank Accession numbers. The phylogram was rooted using *Amphilogia gyrosa* and *Aurapex penicillata*.

Figure 4 (below):
Growth of twelve *Cryphonectria parasitica* isolates at 30 ° C. over a period of seven days. Culture photos of SWC4-7 (bottom) and *C. parasitica*-8 (top).



Conclusions

- The identification of *C. parasitica* represents another incursion of an alien invasive pathogen into the UK.
- Surveys are underway to determine the distribution of the organism.
- Further experiments are underway to isolate and characterise the dsRNA elements associated with putative hypovirulent strains of *C. parasitica*.

References

Biraghi A, 1950. La distribuzione del cancro del castagno in Italia. *L'ItaliaForestale e Montana* 5, 18–21.

Gryzenhout M, Myburgh H, Van derMerwe NA, Wingfield BD, Wingfield MJ, 2004. *Chrysoporthe*, a new genus to accommodate *Cryphonectria cubensis*. *Studies in Mycology* 50, 119–142.

Möller EM, Bahnweg G, Sandermann H, Geiger HH, 1992. A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. *Nucleic Acids Research* 20, 6115–6116.

Robin C, Heiniger U, 2001. Chestnut blight in Europe: Diversity of *Cryphonectria parasitica*, hypovirulence and biocontrol. *Forest Snow and Landscape Research* 76, 361–367.

Sivanesan A, Holliday P, 1981. *Cryphonectria parasitica*. *CMI Descriptions of pathogenic fungi and bacteria*. No. 704, Set. 71. Kew, Surrey, UK: Commonwealth Mycological Institute.

Stamatakis A, Hoover P, Rougemont J, 2008. A rapid bootstrap algorithm for the RAxML web servers". *Systematic Biology* 57, 758–771.