

Supplementary material 2 - DNA extraction and Real-Time PCR assay and the isolation assay: methods used for *Ceratocystis platani* detection in the discoloured wood of surviving trees.

DNA was extracted from wood samples (70 mg), both healthy and altered by fungal infections. They were powdered with liquid nitrogen and the DNA was extracted using a DNeasy Plant Mini Kit (Qiagen, Germany) following the manufacturer's instructions.

The mycelium of the *C. platani* strain used in the inoculation-trials was harvested after being scraped from actively growing cultures using sterile pipette tips. DNA was then extracted as described above.

Real-Time PCR amplifications were carried out on a CFX96 Real-Time PCR Detection System (BIO-RAD) using an EvaGreen assay [SsoFast™ EvaGreen® supermix (BIO-RAD)]. Reactions were performed in 20- $\mu$ l volumes containing 10  $\mu$ l of the supermix cited above, 0.5  $\mu$ M of both forward and reverse primers, and from 2 to 6  $\mu$ l of the template.

*C. platani* genomic DNA (10 ng and 10 pg) and a DNA extracted from an actively spreading *C. platani* canker, were used as positive controls. No-template reactions and reactions containing genomic DNA of *P. × acerifolia* extracted from healthy wood, were used as negative controls.

Primers were: C.P.Sn.For.I CGTACCTATCTTG TAGTGAGATGAATGC, with a forward orientation; C.P.Sn.Rev.I GAGTTTACAGTGGCGAGACTATACTG, with a reverse orientation.

Cycling conditions of Real-Time PCR: initial denaturation at 96°C for 3 min; 40 cycles at 95°C for 10 s (denaturation) and 66°C for 20 s (annealing/extension); final extension at 72°C for 5 min.

Isolation assay: soon after cutting surviving trees, small portions of wood were collected from discoloured areas in proximity of the inoculation point. They were rapidly flamed and plated on Potato Dextrose Agar (PDA) (Oxoid) supplemented with Streptomycin 0.2 gr L<sup>-1</sup>. The plates

were incubated at 25°C in the dark. *Ceratocystis platani* colonies were identified by observing the typical ascospores and conidia under a light microscope.