

The detection of *Ceratocystis fagacearum* in Texas live oak using real-time polymerase chain reaction

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The diagnosis of oak wilt depends on isolation of the pathogen, *Ceratocystis fagacearum*, from wood tissues of symptomatic trees plated onto a growth medium such as acidified potato dextrose agar. This technique is time consuming, inefficient and has many limitations, including a long incubation period which often produces false, negative results. With the objective of developing a specific molecular detection system, 16 GenBank accessions of *Ceratocystis* ITS DNA sequences were used to generate a BLAST alignment to look for regions of variation that could be used to discriminate for *C. fagacearum* in a quantitative real-time PCR assay. Two regions, including 341 to 510 bp (ITS1) and 651 to 820 bp (ITS 2) were selected and further compared with 81 data bases of fungal DNA sequences. Primers and fluorescent labeled probes specific for *C. fagacearum* were designed using Primer Express[®] software (Applied Biosystems). These primer/probe sets successfully detected the pathogen from cultured spore suspensions and purified, target DNA without amplifying closely related, non-target fungal species. The ITS primer/probe set CIP2 consistently detected *C. fagacearum* from sampled, symptomatic trees that were confirmed by pathogen isolation. The preliminary testing of this technique demonstrates the potential for this tool to be a significant breakthrough in the diagnosis of oak wilt and invaluable in the study of this destructive tree pathogen.