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Use of real-time and nested PCR to detect Phytophthora ramorum in infested nursery container mixes and soils

G. Colburn (1), S. Jeffers (2)

(1) Clemson University, Pendleton, SC, U.S.A.; (2) Clemson University, Clemson, SC, U.S.A.

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Phytophthora ramorum has been shown to be present in field soil and container mixes (i.e., substrates) at ornamental plant nurseries. P. ramorum can be recovered from a substrate using a baiting bioassay, which involves culturing of infected bait pieces on selective medium (PARP-V8). Results may not be available for 2-4 weeks. Techniques for the rapid detection of P. ramorum in substrates are needed to give regulators a better tool to prevent the spread of this quarantined pathogen. Two or three replicate aliquots from each of 23 substrate samples were baited with leaf disks from Camellia japonica and Rhododendron catawbiense. Some of the bait pieces from each aliquot then were embedded in PARPH-V8 to isolate P. ramorum, and DNA was extracted from other bait pieces for molecular detection of the pathogen. DNA was examined by both real-time and nested PCR using the ITS gene target following the USDA-APHIS protocol. Of the 23 substrate samples, six were found to be positive for P. ramorum by both real-time and nested PCR and by isolation. In one substrate sample, P. ramorum was detected by nested PCR and not by real-time PCR or isolation. Overall, there was agreement among detection assays for 22/23 substrate samples (96%). Real-time and nested PCR appear to be as reliable as isolation on selective medium for detecting P. ramorum from leaf pieces used as soil baits. In addition, these two detection assays greatly reduce the time required to obtain results.