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[\[PDF \(507K\)\]](#) [\[References\]](#)**Detection of *Pythium helicoides* in flower production area using PCR with species-specific primers.**YIN LING¹⁾, K. KAGEYAMA¹⁾, T. ASANO¹⁾, M. SENDA¹⁾, H. WATANABE²⁾, H. SUGA³⁾ and H. FUKUI⁴⁾

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ABSTRACT

Pythium helicoides is an important pathogen causing root rot of several crops, especially miniature rose and kalanchoe in ebb-and-flow irrigation systems that recycle nutrient solutions. In this study, we developed a PCR detection method with species-specific primers to determine sites inhabited by *P. helicoides*. The primers were designed based on the sequences of the internal transcribed spacer region of rDNA. The specificity of the primers was examined using 34 species and two groups of *Pythium* including *P. helicoides*, and three species in a closely related genus. The amplification with the primers detected only *P. helicoides*, confirming the specificity of the primers. The PCR amplified as little as 100 fg of genomic DNA of *P. helicoides*. When the PCR was used with naturally infested soil, the detection limit ranged from 14 to 35 cfu/g of dry soil. When this detection method was used to investigate location of *P. helicoides* in commercial production of miniature rose and kalanchoe, the pathogen was detected both inside and outside the greenhouses but not in the potting soil mixture, suggesting a different source other than potting soil for pathogen contamination. There are several sources of *P. helicoides* to contaminate clean plant, soil and the recycling nutrient solution during culture.

Key words: *Pythium helicoides*, species-specific primer, PCR detection, life cycle, ebb-and-flow irrigation, hydroponic culture

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