Developing rapid diagnostic assays for the rusts: collections, sequence analyses and PCR-based DNA hybridization techniques

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PCR-based DNA hybridization techniques have been used for the detection of plant pathogenic fungi, nematodes, bacteria, and viruses. Based on sets of diagnostic oligonucleotides (oligos), designed to be specific for the target organisms at the required level of taxonomic resolution, the accuracy of this detection method depends on the availability of a large number of reference specimens in the development stages, and continuing validation and screening for novel genotypes. Parsimony analyses are used to identify clades of closely-related taxa and reveal any potential identification or taxonomic issues to be resolved. A hierarchical system of group-specific oligos diagnostic at multiple taxonomic ranks is designed, capitalizing on the value of single nucleotide polymorphisms, gaps and insertions as unique characters. The oligos are bound to a nylon membrane to which PCR products of unknowns can be hybridized, resulting in a dot if the sample is positive. We are developing such a macroarray detection system for rust fungi, with an initial focus on the cereal rust pathogens. Our primary source of specimens has been several international mycological herbaria, as well as the Canadian collection at AAFC. DNA was extracted from infected leaf samples and rDNA sequences (ITS and 28S) determined from PCR products using rust-specific sequencing primers. Using an ITS gene tree, we demonstrate the potential effectiveness of this approach for differentiating among supra- and sub-specific groups of taxa related to *Puccinia coronata*, *P. graminis* and *P. striiformis*. As data for more rapidly evolving and highly variable gene regions becomes available, this approach may be adapted to detect variation at the population level of resolution.