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# A Survey of *Phytophthora* spp. in Midwest Nurseries, Greenhouses, and Landscapes

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## Abstract

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A survey of nurseries, greenhouses, and landscapes was conducted from 2006 to 2008 in order to determine the prevalence and diversity of *Phytophthora* spp. From sites in Iowa, Michigan, Ohio, and, predominantly, Indiana, 121 *Phytophthora* isolates were obtained from 1,657 host samples spanning 32 host genera. Based on sequence of the internal transcribed spacer (ITS) region of the ribosomal DNA, 11 *Phytophthora* spp. and two hybrid species were identified. A majority of the isolates were *P. citricola* (35.9%) or *P. citrophthora* (27.4%). Six

isolates were confirmed as hybrids (four of *P. cactorum* × *hedraiaandra* and two of *P. nicotianae* × *cactorum*) by cloning and sequencing the ITS region. Three *P. cactorum* × *hedraiaandra* isolates were obtained from the same site, from three *Rhododendron* spp., which are known hosts to the parental species. The fourth isolate, however, was recovered out of a different location in a *Dicentra* sp., which is not a known host to either parental species, suggesting an expansion of host range of the hybrid isolate as compared with either parental species.

*Phytophthora* spp. are important pathogens in many agricultural and ecological systems (5,9,11,17,27) and are major threats to the floriculture and nursery industries, which grow a diversity of species in close proximity to each other in nurseries and greenhouses (9,11,27,32). In these situations, *Phytophthora* spp. can cause significant disease outbreaks, including root rots, crown rots, leaf blights, cankers, and stem dieback (11), jeopardizing the horticultural industry's \$11.7 billion sales value (31).

*Phytophthora* spp. are also of an ecological concern. *Phytophthora ramorum*, the causal organism of sudden oak death (SOD), has caused significant mortality of oak (*Quercus* spp.) and tanoak (*Lithocarpus* spp.), disrupting the forest systems (26). Since its introduction to the United States in 1994, *P. ramorum* has been detected in forests and nurseries in 14 coastal counties of northern California and 1 county in southern Oregon (26). *P. ramorum* has a wide host range consisting of 45 proven plant species and 91 associated plant species (30) and is thought to have a high potential to devastate eastern U.S. forests if ever introduced to these systems (15). There is a significant risk of this disease spreading throughout the United States due to the extraordinary amount of interstate trade of nursery and greenhouse plant species (29). An increase in public concern for invasive plant pathogens has prompted many researchers to survey for native and exotic species of *Phytophthora* and to investigate the risks they impose (5,9,11,27,32). A comprehensive survey of native and endemic *Phytophthora* spp. present in an area will provide tools to more rapidly identify new threats as they develop and, possibly, prevent unnecessary quarantines from being imposed against endemic *Phytophthora* spp. (27).

In order to be successful, surveys of *Phytophthora* spp. require accurate and efficient identification methods. In the past, identification was based solely on morphological traits (23,28,33). However, the variability of morphological characteristics that occur within species, the inconsistency of these structures in culture, and the similarity of characteristics between species can often lead to erroneous identifications (20). Therefore, numerous studies have

used molecular techniques such as restriction fragment length polymorphisms (3), amplified fragment length polymorphisms (3), isozyme analysis (6), single-strand conformation polymorphisms (16), and DNA sequence analysis (6,7,20,21) to aid in identification.

Many of these techniques rely upon sequence variations within the internal transcribed spacers (ITS) of the ribosomal genes, a part of the nuclear DNA that does not code for a protein (34). Mutations in this region have no apparent fitness penalties and, therefore, tend to accumulate quickly (34), which leads to highly variable sequences useful for identification of many *Phytophthora* spp. (3,20). However, Cooke et al. (6) stated that *P. citricola* and *P. inflata* have identical internal transcribed spacer (ITS) sequences, making analysis of other genes necessary to accurately distinguish between these two species. Nevertheless, the ITS region is still an incredibly useful identification tool because the sequence of the ITS region is readily discerned and, as a result, a large number of sequences are available from several public databases (14,18).

Another threat that *Phytophthora* spp. pose is the formation of new and hybrid species. Various studies describe new *Phytophthora* spp. and hybrids, which likely arose from formerly geographically distant pathogen species that lack robust reproductive barriers coming into contact in the same area (4,14,20,21,27). There is a high probability of different *Phytophthora* spp. being moved to close proximity to one another as international plant trade continues to result in the shipment of latently infected plants long distances. This increases exposure of plant production systems and natural ecosystems to invasive pathogens and creates the opportunity for hybridization between previously geographically separated species (5). For example, naturally occurring *P. cactorum* × *nicotianae* (3,22) and *P. cactorum* × *hedraiaandra* (21) hybrids were recently described and characterized as being morphologically similar to *P. cactorum* but with noticeably different genotypes and host ranges. The *P. cactorum* × *hedraiaandra* hybrid was reported to be pathogenic to the plant genera *Allium*, *Idesia*, and *Penstemon*, none of which was a host to either parental *Phytophthora* spp. (21). *P. alni* subsp. *alni* is a naturally occurring hybrid species with a different host range that may have resulted from the multiple hybridization events between *P. alni* subsp. *uniformis* and *P. alni* subsp. *multiformis* (4,23). *P. alni* subsp. *alni* is the first *Phytophthora* sp. reported to be pathogenic on alder (*Alnus* spp.) (4) and is a major threat to European riparian forest systems (13). Due to the diversity and hybridization of *Phy-*

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