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An *in-situ* baiting bioassay for detecting *Phytophthora* species in irrigation runoff containment basins

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Experiments were conducted in irrigation runoff containment basins to assess the effects of bait species (*Camellia japonica*, *Ilex crenata* or *Rhododendron catawbiense*), bait type (whole leaf vs. leaf disc), baiting duration (1, 2, 7 or 14 days), baiting depth and growth media (modified PARP-V8 or PARPH-V8) on the recovery of *Phytophthora* species. A two-rope, flexible bait-deployment system was compared with a one-rope fixed system for bait stability at designated locations and depths. A total of 907 *Phytophthora* isolates were subjected to PCR-based single-strand conformation polymorphism (PCR-SSCP) analysis to identify to species level. Seven distinct SSCP patterns representing six morphospecies: *P. citricola* (Cil I), *P. citrophthora* (Cip I), *P. hydropathica* (Hyd), *P. insolita* (Ins), *P. megasperma* (Meg I & II) and an unidentified *Phytophthora* species were identified. Irrespective of culture medium, 7 days of baiting with rhododendron leaves consistently resulted in the recovery of the greatest diversity and populations of *Phytophthora* species with minimum interference from *Pythium* species. The flexible bait-deployment system was superior to the fixed system, minimizing the risk of bait loss and dislocation of baiting units and allowing baits to remain at designated depths from the surface under inclement weather.

Keywords: baiting assay, PCR-SSCP, *Pythium*, recycling irrigation, *Rhododendron*

Introduction

Recycling irrigation is increasingly important to nursery growers and other crop production enterprises because of diminishing water availability, but this practice potentially recycles and spreads plant pathogens. A large range of plant pathogenic bacteria, fungi, oomycetes, nematodes and viruses has been reported from irrigation water resources, in particular irrigation runoff containment basins (Hong & Moorman, 2005). Specifically, contaminated irrigation water has long been recognized as an important source of inoculum for *Phytophthora* species, a group of destructive pathogens of a wide range of economically and ecologically important plant species (Erwin & Ribeiro, 1996; Gallegly & Hong, 2008).

Phytophthora species are generally regarded as 'water moulds', but their aquatic ecology is largely unknown (Reeser *et al.*, 2007; Hong *et al.*, 2008a). Data on occurrence, distribution and population dynamics of *Phytophthora* species in the aquatic environment are essential to supplement the information available in the terrestrial environment, and to formulate effective disease-management strategies. The first step to obtain such data is to identify and/or develop a bioassay that can consistently and effectively detect a diversity of *Phytophthora* species present in the containment basins.

An array of bioassays has been reported for detecting *Phytophthora* species in irrigation water (MacDonald *et al.*, 1990; Hong *et al.*, 2002; Kong *et al.*, 2003a; Hwang *et al.*, 2006). Each of these assays has its advantages and limitations. For example, filtration methods can generate semiquantitative data, but can usually only process a very limited amount of water compared to the huge body of water in containment basins. As a result, the data obtained may not represent the targeted microbial communities. Immunological and DNA-based techniques are mostly species- or genus-specific, thus they are of limited relevance to studies tracking the dynamics of multiple species of *Phytophthora* in the containment basins. Baiting with leaf discs is commonly used to study *Phytophthora* spp. in

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