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

S. R. Ghimire[a], P. A. Richardson[a], G. W. Moorman[b], J. D. Lea-Cox[c], D. S. Ross[d] and C. X. Hong[a]*

[a]*Hampton Roads Agricultural Research and Extension Center, Virginia Polytechnic Institute and State University, Virginia Beach, VA 23455;* [b]*Department of Plant Pathology, Pennsylvania State University, University Park, PA 16802;* [c]*Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD 20742; and* [d]*Department of Environmental Science and Technology, University of Maryland, College Park, MD 20742, USA*

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

*E-mail: chhong2@vt.edu

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irrigation systems and can potentially recover multiple species of *Phytophthora* (Ferguson & Jeffers, 1999). The wounded perimeters of leaf discs, however, are vulnerable to contaminants, especially *Pythium* (Jeffers & Aldwinckle, 1987; Ferguson & Jeffers, 1999). As *Pythium* spp. are more prevalent in irrigation water and grow more rapidly than *Phytophthora* spp. (Shokes & McCarter, 1979; Ali-Shtayeh & MacDonald, 1991; Bush *et al*., 2003), isolating pure *Phytophthora* cultures as required to identify to species level is often difficult when leaf discs are used as bait.

One possible alternative is the use of whole leaves instead of leaf discs as bait. Whole rhododendron leaves have been successfully used to detect multiple *Phytophthora* spp. from root and soil/substrates, as well as in hydroponic systems (Themann & Werres, 1997, 1998). In an exploratory study for the current work a large number of pure *Phytophthora* isolates were recovered from containment basins baited with rhododendron leaves.

Any baiting assay involves bait choices and selection of deployment system, baiting depth and duration, as well as culture medium. Each of these choices may affect assay quality and productivity. For instance, *in-situ* baiting using *Banksia grandis* seedlings detected *P. cinnamomi* in twice as many areas as *ex-situ* soil and root baiting (McDougall *et al*., 2002). Although a handful of baiting assays have been documented in the past decades, little is known about the effects of bait choice and other baiting components on the recovery of *Phytophthora* species from water. The specific objectives of this study were to (i) evaluate whole leaves and leaf discs of three plant genera as bait choices, (ii) compare 2-, 7- and 14-day baiting with camellia and rhododendron leaves, (iii) study the distribution of *Phytophthora* spp. across the water column, (iv) assess two growth media for selective isolation of *Phytophthora* species, and (v) develop a flexible bait-deployment system that would allow baits to remain at designated locations and depths from the water surface under inclement weather.

Materials and methods

Nursery and water containment basin

An 8-year-old irrigation runoff containment basin occupying an area of 1 ha at a container nursery in eastern Virginia, USA. representing USDA plant hardiness zone 7b (Cathey, 1990), was selected for this study. This nursery used overhead irrigation, once or twice a day for 90 min, depending upon weather conditions. The basin received runoff water through a single entrance from an 8-ha production area growing woody plants: primarily azaleas, camellias, holly, juniper, maple and rhododendron in plastic containers on polyethylene sheets. The amount of runoff water returning to the basin each day was estimated at 511 000 L. The nursery experienced crop losses from *Phytophthora* spp. prior to and during this study (C.X. Hong, unpublished data).

Bait and bait preparation

Plant species included as bait were camellia (*Camellia japonica* cv. Governor Mouton), holly (*Ilex crenata* cv. Japanese Mobjack Supreme) and rhododendron (*Rhododendron catawbiense* cv. Boursault). Leaves were harvested from healthy plants at the Hampton Roads Agricultural Research and Extension Center in Virginia Beach, Virginia, rinsed in tap water and surface-sterilized with 70% ethanol for 30 s, followed by two rinses in distilled water. Halves of camellia and rhododendron leaves were cut into 0·6-cm^2 pieces. Leaf discs were then placed into nylon mesh bags with 40 pieces of each bait species per bag. Two whole leaves each of camellia and rhododendron and 10 leaves of holly were placed directly into a larger mesh bag.

Baiting and bait deployment

The first baiting was performed in November 2005, with additional baiting in January, March and August 2006. Treatment details for each baiting month are summarized in Table 1. Baits were deployed in surface water at the runoff entrance and at three different depths (surface, 0·5 m and bottom) 40 m downstream from the entrance. At each baiting point and depth, triplicate bait sets were placed 1 m apart. A total of six camellia leaves, six rhododendron leaves, 30 holly leaves and 120 leaf discs of each plant species were deployed at each depth. Water temperatures were taken at the time of bait deployment and collection using a digital thermometer A150Q-CERT (Cooper-Atkins) and water quality meter U10 (Horiba STEC, Inc.) in March and August, respectively.

A fixed system of bait deployment was modified to maintain baits at specified positions and depths in the containment basin. A rope was tied to a double-layered nylon mesh bag filled with 2–3 kg of rock pieces at one end. The length of rope was 3 m, long enough to accommodate water-level fluctuations. The other end of the rope was tied to a styrofoam float. Nylon mesh bags with baits were attached to the rope near the float for surface deployment and near the weight for bottom deployment. A separate shorter rope was used to position the baits at intermediate depths. One end of this rope was tied to the float and other end was weighted with a small rock heavy enough to stretch the rope in the water column, but not touch the bottom of the basin (Fig. 1). The stability of baiting units in flexible and fixed bait-deployment systems was monitored in the study basin and three other basins across the Commonwealth of Virginia. Numbers of baiting units displaced, submerged or lost during extreme weather were recorded for both deployment systems.

Isolation and culture preparation

After the specified baiting period, baits were collected and rinsed with tap water, surface-sterilized with 0·5% hypochlorite solution (v/v) for 30 s, and rinsed twice with distilled water. Whole leaves and leaf discs were blotted

Table 1 Treatments and treatment combinations to study effect of baiting components on recovery of *Phytophthora* spp. from an irrigation runoff containment basin in 2005 and 2006

			Baiting duration (days)				Culture medium	
Month of experiment	Bait	Bait type	1	2	7	14	PARP-V8	PARPH-V8
Nov. 2005	Camellia	Leaf	–	+	–	–	+	+
	Holly	Disc	+	–	–	–	+	+
		Leaf	–	+	–	–	+	+
	Rhododendron	Disc	+	–	–	–	+	+
		Leaf	–	+	–	–	+	+
Jan. 2006	Camellia	Leaf	–	+	–	–	+	+
	Holly	Disc	+	–	–	–	+	+
	Rhododendron	Disc	+	–	–	–	+	+
		Leaf	–	+	–	–	+	+
Mar. 2006	Camellia	Leaf	–	+	+	+	+	+
	Rhododendron	Leaf	–	+	+	+	+	+
Aug. 2006	Camellia	Disc	+	–	–	–	+	+
		Leaf	–	+	+	+	+	+
	Holly	Disc	+	–	–	–	+	+
		Leaf	+	+	+	+	+	+
	Rhododendron	Disc	+	–	–	–	+	+
		Leaf	+	+	+	+	+	+

+ and – denote the presence and absence of the corresponding treatments, respectively.

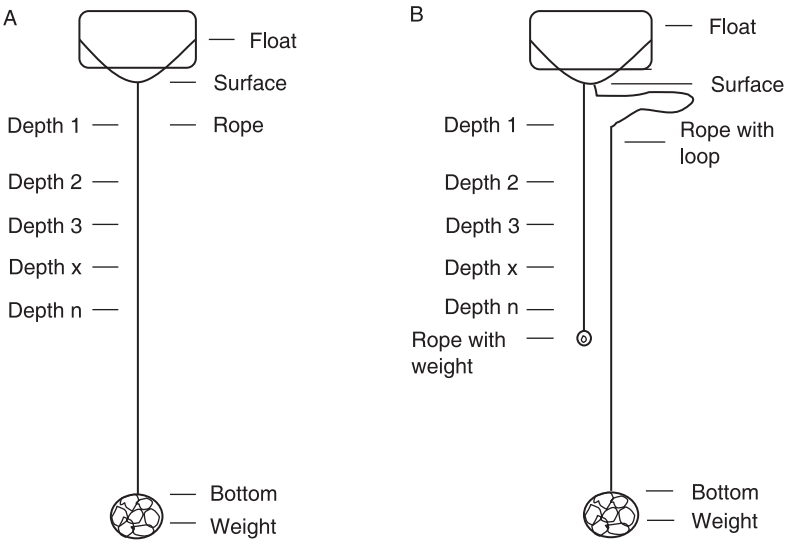

Figure 1 (A) Single-rope, fixed bait-deployment system and (B) two-rope, flexible bait-deployment system. Both systems have a float and a major weight which are connected by a rope. The length of this rope is equal (fixed) to the depth of water column on the day baits are deployed for the single-rope system and all baits are attached to this rope at depths desired. The two-rope system has a longer major rope to allow adjustment to water level rise during baiting periods (flexible) and a second rope with a small weight (barely enough to stretch the rope) to keep all baits except for that at the bottom at the same depths as measured from the surface.

dry on clean paper towels. Whole leaves were cut into small pieces of the same size (0·6 cm^2). Leaf discs were then transferred to 100- × 15-mm Petri dishes (10 discs per dish) with PARP-V8 or PARPH-V8 agar (Ferguson & Jeffers, 1999; Hong *et al*., 2002). Leaf discs of each bait plant species (without exposure to the basin water) were also incubated on PARP-V8 agar as controls. Dishes were incubated in the dark at room temperature. Emerging colonies in each isolation dish were marked by culture morphology as *Phytophthora* or *Pythium*. *Phytophthora* species grew slowly with dense and fluffy mycelium and regular margins, whereas *Pythium* species grew much more rapidly, often quickly spreading over the entire dish. Daily colony counts of both genera were recorded for 5 consecutive days. At least 10% of suspected *Phytophthora* colonies from each sampling month were randomly selected and subcultured on PARP-V8 agar in a multi-well plate for further identification to species level.

Single-strand conformation polymorphism (SSCP) of rDNA

All subcultures were subjected to a direct colony PCR-SSCP assay as described previously (Kong *et al*., 2005). This DNA fingerprinting technique allows species identification (Kong *et al*., 2003b, 2004) and has been incorporated into a new key for *c*. 60 *Phytophthora* species (Gallegly & Hong, 2008). Selected isolates

representing each SSCP pattern were aligned side by side with key isolates (Gallegly & Hong, 2008) of suspected species in the attempt to identify to species level.

Morphological characterization

A small portion of the subcultures representing different SSCP patterns from each sampling date were examined morphologically to confirm their identities. Asexual structures including shape and size of sporangia, papillation, sporangiophore morphology, mycelium type and zoospore differentiation mechanisms were studied. Mating type and growth temperature maximum were also determined as needed. Isolates having a SSCP pattern distinct from known species of *Phytophthora* were initially identified to the genus using sporangium production and zoospore differentiation patterns. *Phytophthora* keys (Waterhouse, 1970; Stamps *et al*., 1990; Bush *et al*., 2006) were used to identify selected cultures to species level.

Statistical analysis

Colony counts of *Phytophthora* species and *Pythium* were divided by the total number of leaf discs placed in each dish to convert the numerical data to percentages (i.e. percentage of leaf discs colonized). The percentage data were subjected to arcsine transformation to normalize the distribution. Transformed data were analysed within the framework of general linear models using PROC GLM (SAS Institute). Duncan's multiple range tests were performed to compare the effects of different baiting components on the recovery of *Phytophthora* and *Pythium* spp. *Phytophthora* recovery data from the different depths of water column 40 m from the entrance were also subjected to similar analysis.

Results

Hyphal growth was not observed in dishes with leaf discs that were not exposed to the basin water. In contrast, a total of 4249 *Phytophthora* colonies were recovered from 17 310 baits retrieved from the runoff containment basin during the study period. Mean water temperatures ranged from 6 to 13°C in March and increased to 26 to 30°C in August (Table 2).

Table 2 Water temperature (°C) in an irrigation runoff containment basin on days of bait deployment and retrieval in March and August 2006

		March				August			
Distance	Depth	1	3	7	14	7	9	14	21
Entrance	Surface	5·9	9·2	12·0	12·9	29·8	31·3	26·1	28·0
40 m	Surface	5·9	9·6	10·5	13·0	30·0	30·3	26·2	28·3
	0·5 m	6·0	9·6	8·4	12·9	30·0	30·3	25·9	28·4
	Bottom	6·0	9·6	8·4	12·9	29·6	30·1	25·6	28·3
Mean	–	5·9	9·5	9·8	12·9	29·9	30·5	26·0	28·3

SSCP fingerprinting and morphological confirmations

The SSCP analysis of 907 isolates detected seven distinct fingerprints belonging to six species, as confirmed by morphological examination. These species were *P. citricola* (Cil I), *P. citrophthora* (Cip I), *P. hydropathica* (Hyd), *P. insolita* (Ins), *P. megasperma* (Meg I & II) and an unidentified species (Kong *et al*., 2003b; Gallegly & Hong, 2008; Hong *et al*., 2008b). Isolates of this unknown species produce ellipsoid to obpyriform, nonpapillate sporangia, a swollen base in the sporangiophore, and hyphal swellings and hyphal aggregation in clusters. They grew at an average rate of 1·5 mm day^{-1} at 35°C. Mating of these isolates with testers of *P. meadii* and *P. nicotianae* for 4 months did not produce any oospores.

Baits and bait types

Whole-leaf baits overall were superior to the corresponding leaf discs and yielded high numbers of pure *Phytophthora* colonies irrespective of bait plant species evaluated. The highest numbers of *Phytophthora* colonies were recovered in November from rhododendron leaf discs (50%), followed by rhododendron leaves (28%), camellia leaves (20%), holly leaves (9%) and holly leaves disc (2%). Accompanying the highest recovery of *Phytophthora* from rhododendron leaf discs was an extensive *Pythium* colonization (96%). Comparatively, more *Phytophthora* than *Pythium* colonies were recovered from whole leaves of camellia and rhododendron. In January, the recovery ratio of *Phytophthora* to *Pythium* was 2:1 for camellia and rhododendron leaves, 1:5 and 1:6 for rhododendron and holly leaf discs, respectively. Similar recovery ratios were observed in August (data not shown).

More species of *Phytophthora* were recovered from whole-leaf than leaf-disc baits (Table 3). Specifically, the highest diversity was detected with rhododendron leaves, with three morphospecies in November, and two DNA fingerprint types in January. In November *P. megasperma* (Meg I) was a dominant species, accounting for 94% of the total recovery, while *P. citrophthora* and *P. citricola* accounted for 5% and 1%, respectively. *Phytophthora megasperma* was the only species detected in January with two SSCP patterns: Meg I (99%) and Meg II (1%). The least diversity was detected with camellia leaf discs. Similar observations were made in August (Table 3).

Baiting duration

The numbers of *Phytophthora* species and colonies recovered increased with extension of baiting period (Table 3). As baiting period extended from 2 to 14 days in March, the recovery of *Phytophthora* species from rhododendron leaves increased from 5 to 28%. However, the highest recovery of *Phytophthora* species was 74% from rhododendron leaves with a 7-day exposure in August. Baiting for 14 days increased *Pythium* colonization and reduced *Phytophthora* colonization. A similar association was observed in baiting with camellia leaves (data not shown).

Table 3 Effect of bait type and baiting duration on the diversity and recovery of *Phytophthora* spp. from an irrigation runoff containment basin in the different months of 2005 and 2006

		Diversity and recovery in different baiting months[a]			
Bait	Duration (days)	Nov. 2005	Jan. 2006	Mar. 2006	Aug. 2006
Camellia disc	1	–[b]	–	–	0 (None)
Camellia leaf	2	2 (Cip I, Meg I)	2 (Meg I, Meg II)	2 (Meg I, Meg II)	2 (Hyd, *Phytophthora* sp.)
	7	–	–	1 (Meg I)	1 (Hyd)
	14	–	–	2 (Meg I, Meg II)	3 (Hyd, Ins, *Phytophthora* sp.)
Holly disc	1	1 (Meg I)	2 (Meg I, Meg II)	–	1 (Hyd)
Holly leaf	1	–	–	–	1 (Hyd)
	2	2 (Cil I, Meg I)	–	–	1 (Hyd)
	7	–	–	–	1 (Hyd)
	14	–	–	–	1 (Hyd)
Rhododendron disc	1	1 (Meg I)	1 (Meg I)	–	2 (Hyd, *Phytophthora* sp.)
Rhododendron leaf	1	–	–	–	2 (Hyd, *Phytophthora* sp.)
	2	3 (Cil I, Cip I, Meg I)	2 (Meg I, Meg II)	2 (Meg I, Meg II)	2 (Hyd, *Phytophthora* sp.)
	7	–	–	2 (Meg I, Meg II)	3 (Hyd, Ins, *Phytophthora* sp.)
	14	–	–	2 (Meg I, Meg II)	2 (Hyd, *Phytophthora* sp.)
Species recovered		3 (Cil I, Cip I, Meg I)	2 (Meg I, Meg II)	2 (Meg I, Meg II)	3 (Hyd, Ins, *Phytophthora* sp.)

[a]Number of *Phytophthora* species recovered with their DNA fingerprint IDs listed in parentheses: Cil I represents a subgroup of *P. citricola*; Cip I for *P. citrophthora*; Hyd for *P. hydropathica*; Ins for *P. insolita*; Meg I & II for two subgroups of *P. megasperma*.
[b]Absence of the corresponding treatment.

Table 4 Percentage of baits colonized by *Phytophthora* spp., with number of species in parentheses, from different depths of water column at 40 m distance in an irrigation runoff containment basin in different months of 2005 and 2006[a]

Depth	Nov. 05	Jan. 06	Mar. 06	Aug. 06
Surface	12·2 b (2)	1·5 b (1)	10·6 a (2)	14·6 b (2)
0·5 m	14·6 ab (3)	5·8 a (1)	6·2 b (2)	13·9 b (3)
Bottom	19·6 a (2)	6·0 a (2)	9·2 ab (1)	20·5 a (3)
Mean	15·5	4·4	8·6	16·3

Values in columns with different letters differed significantly at 95% confidence level.

Camellia and rhododendron leaf baits detected the same species (*P. hydropathica* and an unknown) within 2 days. *Phytophthora insolita* was an additional species detected by rhododendron with a 7-day baiting and by camellia with a 14-day baiting (Table 3).

Baiting depth

Phytophthora recovery data showed significant differences in the distribution of this organism across the water column in all baiting months (Table 4). Differences were also noticed for the associated species diversity. *Phytophthora* recovery was highest at the bottom of the basin, while recovery from the two upper depths (surface and 0·5 m) was variable in different baiting months.

Growth media

There was no difference in the recovery of *Phytophthora* ($P = 0{\cdot}066$) and *Pythium* species ($P = 0{\cdot}267$) between PARPH-V8 and PARP-V8 agar. Both media detected a similar level of diversity of *Phytophthora* spp. (data not shown).

Bait deployment

There were several occurrences of inclement weather during the study period. Most cases were rain storms which elevated the water levels in the containment basins. Baiting units deployed under the fixed system were highly vulnerable to raised water levels, and the majority of the baiting units (42–53%) were either dislocated or missing. These problems were not encountered with the baiting units deployed in the flexible system.

Discussion

This study improved an existing bait-deployment method for *in-situ* baiting in containment basins and demonstrated that bait choices, placement depth and exposure time directly affect the recovery of *Phytophthora* species. The present research was purposely performed in a containment basin with water quality fluctuation range and pattern typical of irrigation reservoirs at ornamental nurseries in the USA (unpublished data) and in three major seasons throughout the year. Thus, the results of present study will have some important general and practical implications for detecting these pathogens in irrigation systems and for investigations into their aquatic ecology.

The flexible bait-deployment system (Fig. 1) developed in this study is a reliable instrument for studying horizontal and vertical distributions of *Phytophthora* species in containment basins. Since its establishment in 2005, this deployment method has been routinely used in several research projects investigating pathogen aquatic ecology. No baits have been lost, nor has any dislocation and

submergence of baits been encountered. Application of this system has improved the quality and productivity of the research projects and it should be applicable to research into other pathogens and in different geographic locations.

Whole rhododendron leaves were the best bait among the six bait-plant species and type combinations assessed in this study. Rhododendrons tend to be susceptible to many *Phytophthora* species they encounter (Erwin & Ribeiro, 1996; Farr *et al*., 2008; Gallegly & Hong, 2008). The genus is generally regarded as a 'universal suscept' (Brasier, 2004). Thus, it is not unexpected that use of rhododendron baits resulted in the greatest recovery of *Phytophthora* species during this study. Irrespective of bait plant species tested, whole-leaf baits were superior to the corresponding leaf discs. Similar observations were documented previously (Dance *et al*., 1975; Linderman & Zeitoun, 1977; Jeffers & Aldwinckle, 1987; Ferguson & Jeffers, 1999). This superiority may be due the result of restricted access to whole leaves by *Pythium* and bacteria, which are normally not as aggressive as *Phytophthora* species (Werres *et al*., 1997).

Among the four baiting durations assessed, the 7-day exposure appeared the best, resulting in the greatest recovery of *Phytophthora* species without overwhelming competition from *Pythium* spp. Baiting for 2–14 days with whole rhododendron leaves was used successfully to recover multiple *Phytophthora* species from root and soil samples in the laboratory (Themann & Werres, 1998). In the present study, extending the baiting period from 2 to 7 days increased bait colonization by both *Phytophthora* and *Pythium* spp. Further extension increased the recovery of *Pythium* spp., but not of *Phytophthora* spp. This could be a result of *Pythium* spp. outcompeting *Phytophthora* spp. during the extended exposure. Mycelium of the former covered entire isolation dishes plated with leaf discs from the 14-day exposure much sooner than those with discs from shorter exposure periods, which made it more difficult to obtain pure cultures of *Phytophthora* species necessary for further identification to species level. This could be another reason why significantly more *Phytophthora* species were not recovered when baiting duration was extended from 7 to 14 days during this study.

Using the direct colony PCR-SSCP assay (Kong *et al*., 2005) enables identification all the subcultures resulting from each seasonal water sampling in a timely fashion, which was essential to this study. Identifying cultures of *Phytophthora* species using traditional morphological criteria alone is becoming increasingly difficult and in many cases is either impractical or liable to be inaccurate (Brasier *et al*., 2003). This was particularly true for the present study, as *P. megasperma*, *P. insolita*, *P. hydropathica*, and the unknown species recovered were morphologically similar. With the colony PCR-SSCP assay it was possible to amplify the target DNA fragment directly from subcultures (without having to extract the DNA) and determine the identities of all subcultures with few requiring a re-run. Morphological examination of selected cultures was an essential step to confirm the identity of each culture as determined by its DNA fingerprint. As a result, all resultant subcultures were identified from each sampling within a few days, enabling adjustments to the work plan which contributed to the quality and productivity of the research.

In summary, an effective and efficient *in-situ* bioassay for detecting *Phytophthora* species in irrigation runoff containment basins consists of the following components: (i) whole rhododendron leaves as bait, (ii) the flexible bait-deployment system as an instrument, (iii) 7 days exposure time, (iv) transferring baits to either selective medium (PARP-V8 or PARP-H-V8), (v) use of the colony PCR-SSCP as a screening tool to group and identify resulting colonies to species level, and (vi) morphological examination of selected cultures representing different SSCP patterns to confirm their identities. Since 2005 this protocol has been used to investigate and compare horizontal and vertical distribution patterns of *Phytophthora* species in containment basins in the mid-Atlantic region of the USA (e.g. Virginia, Maryland and North Carolina), (Ghimire *et al*., 2006). This protocol can be used to detect *Phytophthora* species in streams and rivers, but water quality parameters are likely to be different from irrigation runoff containment basins.

Diversity and populations of *Phytophthora* species in containment basins vary with time, location and the depth of the water column where baiting assays are performed. This is not unexpected. For example, bait colonization by both genera was higher in August than March, perhaps because of higher water temperatures (Table 2) and possibly elevated levels of nutrients in water containment basins as a result of frequent irrigation in summer months. A total of six species (*P. citricola*, *P. citrophthora*, *P. hydropathica*, *P. insolita*, *P. megasperma* and an unknown) were recovered in this study. This is comparable to the number of species recovered previously from single reservoirs in Virginia (Bush *et al*., 2003) and California (MacDonald *et al*., 1994), but differs in species structure from those previous studies. This difference was mostly caused by the range and number of plant species growing at the individual nurseries and their geographical locations. Most importantly, these are pathogenic to a range of plant species including ornamental plants (Erwin & Ribeiro, 1996; Farr *et al*., 2008; Gallegly & Hong, 2008; Hong *et al*., 2008c). Detection of these *Phytophthora* species in the irrigation water again underlines the great potential for dissemination of these pathogens through the recycled irrigation waters to initiate disease epidemics in horticultural nurseries. This study supports previous findings on the variable distribution of *Phytophthora* spp. in water columns (Bush *et al*., 2003; Ghimire *et al*., 2006) and suggests the need for monitoring *Phytophthora* spp. across water columns.

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