Fluctuations of *Phytophthora* and *Pythium* spp. in Components of a Recycling Irrigation System

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ABSTRACT

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Stringent standards of water quality have prompted many horticultural enterprises to limit pollutant discharge associated with nutrient and pesticide applications. Collecting and recycling effluent is a method that has been implemented by many operations to contain pollutants; however, plant pathogens may be spread through recycled effluent. In this study, *Phytophthora* and *Pythium* spp. present in a water-recycling irrigation system at a perennial container nursery in southwestern Virginia were characterized using filtering and baiting techniques with two selective media. Members of *Phytophthora* were identified to species, whereas *Pythium* spp. were identified to genus only. *Pythium* spp. were recovered more frequently and in greater numbers than *Phytophthora* spp. *Phytophthora capsici, P. citricola, P. citrophthora, P. cryptogea, P. drechsleri,* and *P. nicotianae* were recovered in filtering assays. Only *P. cryptogea* and *P. drechsleri* were identified from baits placed on the surface of the irrigation reservoir, whereas *P. cactorum, P. capsici, P. citricola, P. citrophthora, P. cryptogea,* and *P. drechsleri* were recovered at depths, specifically at 1 and 1.5 m. This research provides data for development of detection technology and management practices for plant pathogens in irrigation water and may lead to improvements in conventional assay protocols.

Additional keywords: chlorination, recycled irrigation water

Many horticultural operations have implemented collection and reuse of effluent water to reduce release of pollutants into the environment outside of the operation. This practice also conserves increasingly costly and scarce water. This method involves collecting nursery effluent into holding ponds until needed for irrigation. In most cases, the recycled effluent is pumped to an irrigation reservoir where it is mixed with fresh water (e.g. river, well, and so on) before use in irrigation. One risk of recycling nursery effluent is the spread of plant pathogens through the irrigation system. Many studies have shown a positive correlation between irrigation with plant pathogen-contaminated water and plant disease (9,16,20,35). MacDonald et al. (18) demonstrated that recycled irrigation water can harbor significant levels of fungal propagules which, when used over time to irrigate crops, resulted in contamination of container crops or root colonization by members of the family *Pythiaceae.*

Zoosporic fungi are the most common fungi occurring in water (2) and the zoosporic genera *Phytophthora* de Bary and *Pythium* Pringsh. contain many plant-pathogenic species. Many species of *Phytophthora*, *Pythium* (8,17,21,24,25), and other plant pathogens (11,25,27,31) have been recovered from irrigation water. *Phytophthora* spp. recovered in water assays include: *P. cactorum* (Lebert & Cohn) J. Schröt. (20,36), *P. cambivora* (Petri) Buisman (20), *P. cinnamomi* Rands (17,19,23,34), *P. citricola* Sawada (19,20,34,36); *P. citrophthora* (R.E. Sm. & E.H. Sm.) Leonian (1,16,19,31,34,35), *P. cryptogea* Pethybr. & Lafferty (1,3,17,19,30,34), *P. gonapodyides* (Petersen) Buisman (24), *P. megasperma* Drechs. (19,20,23,30), *P. nicotianae* Breda de Haan (synonym, *P. parasitica* Dastur) var. *parasitica* (1), *P. palmivora* (E.J. Butler) E.J. Butler (1), *P. parasitica* (3,16,17,19,31,34), and *P. syringae* (Kleb.) Kleb. (16). *P. cinnamomi* also has been reported in natural waterways in Hawaii (15) and in the Southwestern Cape Province of South Africa (32). *P. cryptogea* frequently was isolated from surface waters used to irrigate citrus in the West Bank of Jordan (1,30). Yamak et al. (36) recently analyzed internal transcribed spacer 2 regions to group *Phytophthora* isolates recovered from baits in irrigation water and delineated nine clades, of which five have reference isolates of *P. gonapodyides, P. parasitica*, *P. cactorum*, *P. citricola*/*capsici* Leonian, and *P. cambivora*/*pseudotsugae* P.B. Hamm & E.M. Hans.

Filtering (19,21,34) and baiting (17,34,36) commonly are used for detection of plant pathogens in irrigation water. These techniques are labor intensive but allow species separation, and filtering also allows quantification (13). Commercial immunoassay kits also have been assessed for use in water assays; however, they are prone to cross-reactions between genera and demonstrate limitations in quantification (19).

Several studies have been performed in the United States, using either filtering or baiting techniques, to identify *Phytophthora* and *Pythium* spp. present in recycled irrigation water used for ornamental plant production. *Phytophthora citrophthora, P. citricola, P. cinnamomi, P. cryptogea, P. megasperma, P. parasitica,* and *P. syringae* were isolated by filtering assays from nursery effluent in California (19). *P. cinnamomi, P. cryptogea, P. parasitica,* and *Pythium* spp. were recovered by baiting assays in North Carolina (17). Similar assays were conducted in Oklahoma (34) and Pennsylvania (21).

Prior to this work, no information on plant pathogens in irrigation water in Virginia was available. Industry concerns of increased disease incidence after implementation of recycling irrigation water, in addition to requests for control recommendations for waterborne plant pathogens from Virginia growers, prompted this work. To address these needs and prioritize development of practical tools for rapid detection of waterborne plant pathogens, identification of these organisms and their relative abundance in components of a recycled irrigation water system was necessary.

The objectives of this work were to quantify levels of *Phytophthora* and *Pythium* spp. present in the components of a recycling irrigation system at a perennial container nursery in southwestern Virginia over a 2-year period and to identify the range of *Phytophthora* spp. present. Specifically, occurrences of *Phytophthora* and *Pythium* spp. were determined for three water sources: chlorinated irrigation water, nonchlorinated irrigation water, and nursery effluent; and at three depths: 0, 1, and 1.5 m from the surface of an irrigation reservoir. Both filtering and baiting were used, along with two media selective for *Pythium* and *Phytophthora* spp.

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MATERIALS AND METHODS

Location of sampling and description of the irrigation recycling system. The nursery where water assays were performed is located in southwestern Virginia. In all, 31 acres are used for container production and 3 acres for field production. Annual production is approximately 4 million container perennials, which consist of over 1,800 varieties. Prior to these assays, the nursery had implemented a recycling irrigation water system. This recycling system involves collection of nursery effluent into French drains, where the effluent travels to a holding pond located at the lowest elevation of the nursery property. As irrigation water is needed, the water collected in the holding pond is pumped into the irrigation reservoir, along with river water. The soil-bottom irrigation reservoir is surrounded by cement or rocks on its circumference and is maintained free of vegetation. Water in the irrigation reservoir is not treated with chlorine until it is needed for irrigation; at that point, the water is injected with chlorine prior to being pumped to irrigation risers, which are located at various points throughout the nursery. The initial system used liquid chlorine and was implemented in 1997; however, in August 2000, a chlorine-gas injection system replaced the liquid-chlorine injection system to improve delivery of chlorine to irrigation water during low-flow conditions (e.g., when low volumes of water are being pumped through the irrigation system). Collected and recycled water (i.e., water from holding ponds) meets approximately 50% of the nursery's irrigation requirements.

Filtering assays. Nursery effluent, nonchlorinated, and chlorinated irrigation waters were assayed monthly during 2000 and 2001, except in instances where water was frozen or when there was no nursery runoff. Nursery effluent was collected at the point of entry into the holding pond. Nonchlorinated water was collected from an irrigation riser that delivers nonchlorinated water pumped from the irrigation reservoir. Chlorinated water was collected from an irrigation riser, which delivers chlorine-treated water from the irrigation reservoir. These risers are used in application of irrigation water to crops.

Three 1-liter samples were collected from each source at monthly intervals. Each 1-liter sample was collected in three aliquots, which were taken at least 15 min apart. Temperature, pH, and conductivity were recorded for all 1-liter samples immediately after collection with a Watercheck pH meter (Hanna Instruments, Woonsocket, RI). Free chlorine in samples collected from chlorinated irrigation water was measured with a portable chlorine colorimeter. Water samples were kept cool during transport to the laboratory in an ice chest, where they were insulated from direct contact with ice packs by two layers of cardboard. Samples were processed the same day.

A filtering assay, adapted from MacDonald et al. (19) with some modifications, was used to quantify the populations of *Phytophthora* and *Pythium* spp. present in the 1-liter samples. A magnetic stir bar was spun on a magnetic plate for at least 60 s to suspend propagules in each sample; then, three 50-ml aliquots were removed from each sample for replicate filtering. A 300-ml Gelman filtering apparatus (Pall Gelman Laboratory, Ann Arbor, MI) was used with a vacuum pump to filter samples. From January 2000 through March 2001, 47-mm Nucleopore filters with 3.0-µm pores (Whatman Corp., Ann Arbor, MI) were used; from April 2001 through December 2001, 47-mm Durapore filters with 5.0-µm pores (Millipore Corp., Bedford, MA) were used. The change to Durapore filters was made after investigations revealed increased sensitivity in quantifying pythiaceous fungi and reduction in filtering time (13). Filters from sample aliquots were placed into individual sterile test tubes containing 6 ml of 0.09% agar suspension and vortexed for at least 1 min. While gently agitating the test tube to suspend propagules, 1 ml of the solution was transferred onto two 100-by-15-mm petri dishes each of P_5ARP agar, which is selective for members of the family *Pythiaceae,* and P_5ARP amended with hymexazol (Tachigaren, 70% a.i.; Sankyo Co., Tokyo) at 50 ppm for selection of *Phytophthora* spp. (P_5ARP+H; 14). Both media were amended with benomyl (10 mg/liter) to enhance selectivity (23) and will be referred to henceforth as P_5ARP+B and P_5ARP+B+H. The solution was spread with a sterile glass rod in the petri dishes, then incubated in the dark at 25°C.

The isolation dishes were examined daily for colony growth and the number of pythiaceous colonies was noted over a period of at least 7 days. A minimum of 15% of the colonies was selected arbitrarily and cultured on P_5ARP-V8 agar, in which 20% clarified V8 juice replaced cornmeal as the basal medium. *Pythium* isolates were identified in this study to genus only, but *Phytophthora* isolates were identified to species. Identification to species was accomplished by observing morphological characteristics of asexual and sexual structures, along with observation of growth-temperature maxima. Several *Phytophthora* keys were used in this work (7,12,22,26,33; M. E. Gallegly, *unpublished key*).

Asexual structures necessary for identification were induced by transferring mycelial plugs from the growing edge of colonies on P_5ARP-V8 agar to 60-by-15-mm petri dishes containing nonsterile soil extract or subjecting the transferred mycelial plugs to a mineral salts washing regime. Soil extract was prepared by bringing 15 g of soil to 1 liter with distilled water and mixing on a magnetic stir plate overnight. Soil was allowed to settle in the container at least 24 h before use. The mineral salts solution was prepared as previously described by Chen and Zentmyer (6). The mineral salts washing regime was performed as follows: (i) mycelial plugs in petri dishes were flooded with 10 ml of mineral salts solution; (ii) the wash solution was removed and 10 ml of mineral salts solution was added to the culture, which was allowed to rest at least 10 min at room temperature before removal of the mineral salts solution; (iii) cultures were flooded with another 10 ml of mineral salts solution and incubated at room temperature under 40-W fluorescent lights overnight; and (iv) the mineral salts solution was replaced with 10 ml of fresh mineral salts solution. With both the soil extract or mineral salts regime, cultures were allowed to incubate under 40-W fluorescent lights for 1 to 4 days at room temperature and observed for sporangia production.

Cultures incubating in the dark on P_5ARP-V8 agar were observed for development of sexual structures. Plates were observed at approximately 1- and 2-month intervals for sexual structures and, if none were observed, the isolate was presumed heterothallic. For heterothallic *Phytophthora* spp., pairing experiments with selected isolates were made with A1 and A2 mating types of *P. capsici, P. cinnamomi,* and *P. parasitica* on 20% clarified V8 agar.

CFU/liter were calculated by correlating the proportion of a genus or species identified from the transferred isolates with the entire number of colonies recovered in sampling.

Baiting assays. Baiting was performed in the irrigation reservoir, where water from the river and the nursery effluent holding pond is pumped and held until needed for irrigation. As water is needed for irrigation, water is pumped out of the irrigation reservoir and treated with chlorine by injection prior to delivery to irrigation risers, which are used in delivery of irrigation water to crops. Twenty-four leaf disks (5 mm in diameter) of *Rhododendron catawbiense* Michx. were placed in individual plastic mesh bags and attached to floats on the surface of the irrigation reservoir at monthly intervals. Floats were anchored in place. Baits were placed at three arbitrarily chosen locations around the periphery of the pond at least 2 m from shore. Water temperature, conductivity, and pH were recorded at placement and collection. Baits were collected approximately 48 h after placement and kept cool in an insulated container during transport to the laboratory, where samples were processed the same day. During December 2000 and January 2001, baiting assays were not performed, because the reservoir was frozen. Baiting at two additional depths (1 and 1.5

m) also was performed at quarterly intervals from May 2000 to November 2001. For baiting at depths, plastic mesh bags containing rhododendron leaf disks were attached to a rope along with weights at 1- and 1.5-m intervals below the water surface and suspended with an anchored float at the pond surface.

After collection from the irrigation reservoir, leaf disks were washed with tap water for at least 15 min. Next, leaf disks were blotted dry on clean paper towels and plated on three dishes each of the two selective media in 100-by-15-mm petri dishes for each baiting location, using four leaf disks per dish. Isolation dishes were examined daily for colony growth for at least 1 week. Colonies were counted and transferred, and identification and storage procedures were performed as outlined above for filtering assays. The percentage of leaf disks colonized by *Phytophthora* and *Pythium* spp. was calculated separately for each sample.

Statistical analyses. Statistical analyses were performed using JMP software (2nd ed.; SAS Institute, Inc., Cary, NC). For filtering assays, analysis of variance was performed to compare recovery of *Pythium* and *Phytophthora* spp. on the two selective media. Recovery comparison of *Pythium* spp. among the three water sources (chlorinated, nonchlorinated, and effluent) was done with the Kruskal-Wallis *H* test. However, no comparison was made for *Phytophthora* spp. due to their infrequent recovery. For baiting assays, each month was treated as a sample unit and the Sign test was used to compare the percentage of leaf disks colonized by *Phytophthora* and *Pythium* spp. on the two selective media.

RESULTS

Filtering assays. Water temperature readings at the time of sampling ranged from 3 to 28, 1 to 25, and 1 to 29°C in effluent, nonchlorinated, and chlorinated water, respectively, with respective means of 16, 17, and 15°C. Readings of pH ranged from 6.1 to 8.1, 6.5 to 8.9, and 4.2 to 8.5 in effluent, nonchlorinated, and chlorinated water, respectively, with respective means of 6.9, 7.1, and 7.7. Conductivity measured highest in effluent, ranging from 138 to 414 µS with a mean of 246 µS. Conductivity ranged from 77 to 195 µS in nonchlorinated water and 47 to 213 µS in chlorinated water, with respective means of 118 and 141 µS. Free chlorine levels measured from chlorine-treated water at sample collection were similar in the 2 years, ranging from 0.1 to 3.5 ppm in 2000 and 0.1 to 2.8 ppm in 2001. In both years, mean chlorine levels measured at sampling were 0.6 ppm.

A diversity of *Phytophthora* spp. was recovered from effluent water. *P. citrophthora* was recovered from effluent water during July and September 2000 on both media. Recovery of *P. citrophthora* on P_5ARP+B was 85 CFU/liter in July 2000 and 28 CFU/liter in September 2000 and 47 and 16 CFU/liter, respectively, on P_5ARP+B+H during the same months. *P. citrophthora* and *P. nicotianae* were recovered only on P_5ARP+B+H in August and September 2001, respectively, at a relatively low level of 7 CFU/liter. Recovery during single months from nursery effluent occurred with *P. capsici* and *P. cryptogea* in September and June 2000, respectively, and *P. citricola* in June 2001.

P. drechsleri Tucker was the only member of *Phytophthora* recovered from chlorinated irrigation water during filtering assays in both years, other than an unidentified *Phytophthora* sp. which was recovered in a single instance on P_5ARP+B in 2001.

Recovery from nonchlorinated irrigation water showed more diversity of *Phytophthora* spp. than recovery from chlorinated irrigation water and nursery effluent. Recovery of *P. nicotianae* occurred in a single month during 2000 on P_5ARP+B, while *P. cryptogea, P. citricola,* and *P. drechsleri* were recovered in single months in 2001 on P_5ARP+B. The highest level of *Phytophthora* isolate recovery from nonchlorinated water occurred during November 2001, with recovery of *P. cryptogea* at 54 CFU/liter. On P_5ARP+B+H medium, *P. cryptogea* was recovered in November 2000 and in June and July 2001. *P. drechsleri* was recovered during July and October 2001 on P_5ARP+B+H medium. *P. citricola* and *P. citrophthora* were recovered in May and June 2001, respectively, on P_5ARP+B+H medium.

No difference was observed in total recovery of *Phytophthora* isolates between P_5ARP+B and P_5ARP+B+H media from chlorinated water ($P = 0.22$), nonchlorinated water ($P = 0.48$), and effluent ($P = 0.73$) (Fig. 1). However, as expected, significantly fewer isolates of *Pythium* were recovered on P_5ARP+B+H than on P_5ARP+B media from effluent ($P = 0.02$), nonchlorinated ($P < 0.001$), and chlorinated water ($P = 0.04$) (*data not shown*). Therefore, the following analyses of the filtering assays will focus on recovery of isolates on P_5ARP+B medium.

During colder periods of the years when crops were dormant in the nursery, members of *Pythiaceae* were not recovered from the three water sources (Fig. 2). Overall, *Pythium* spp. were recovered more frequently and in greater numbers than *Phytophthora* spp. from chlorinated and nonchlorinated irrigation water and nursery effluent (Fig. 2).

Pythium spp. were isolated from March through October 2000 from chlorinated irrigation water (Fig. 2A). During sampling in 2001, *Pythium* isolate recovery from chlorinated irrigation water occurred only twice at relatively low levels. *Phytophthora* isolates were recovered in July 2000 and no subsequent recovery occurred until fall 2001, when relatively low levels of *Phytophthora* isolates were recovered from chlorinated irrigation water.

Pythium isolate recovery from nonchlorinated irrigation water fluctuated widely over the 2-year study (Fig. 2B). *Phytophthora* isolates were recovered less frequently and at lower levels compared with *Pythium* isolates from nonchlorinated irrigation water in filtering assays on P_5ARP+B medium. *Phytophthora* isolate recovery levels from nonchlorinated water during the 2 years was infrequent.

Pythium isolate recovery from nursery effluent fluctuated less compared with recovery from chlorinated and nonchlorinated irrigation water, and recovery levels were often much higher (Fig. 2). Recovery of *Pythium* isolates from nursery effluent began in both years during early spring and tapered off in early fall, coinciding with crop dormancy. Recovery of *Pythium* isolates among the three water sources (i.e., chlorinated, nonchlorinated, and nursery effluent) was significantly ($P < 0.01$) different (Fig. 2). *Phytophthora* isolate recovery from nursery effluent in filtering assays on P_5ARP+B medium occurred only twice

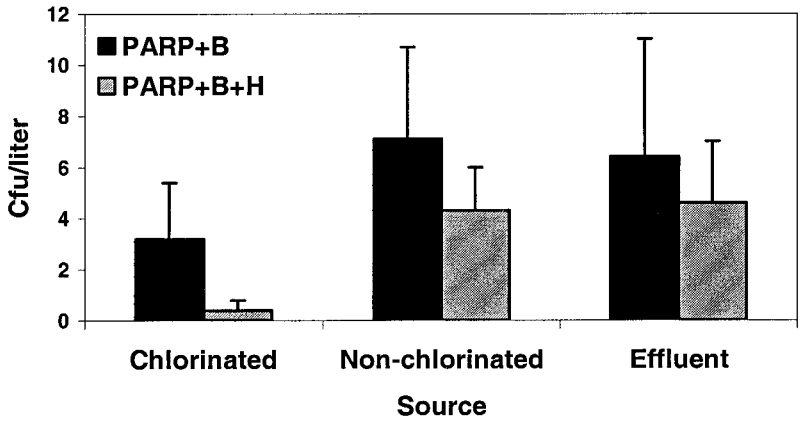

Fig. 1. Total recovery of *Phytophthora* spp. on P_5ARP agar plus benomyl (P_5ARP+B) and P_5ARP+B amended with hymexazol (P_5ARP+B +H) media from chlorinated and nonchlorinated irrigation water, and nursery effluent during monthly filtering assays during 2000 and 2001. Error bars represent + 1 standard deviation.

in the first year of the study; no recovery of *Phytophthora* spp. occurred during 2001 on P_5ARP+B.

Baiting assays. The temperature of water in the irrigation reservoir ranged from 3 to 31°C, with a mean temperature of 16°C. Readings of pH ranged from 6.5 to 8.5 with a mean of pH 7. Conductivity readings ranged from 82 to 188 μS with a mean of 117 μS.

Phytophthora spp. were recovered from significantly ($P = 0.001$) more baits placed on the surface of the irrigation reservoir, using P_5ARP+B+H compared with P_5ARP+B medium (Fig. 3A). In contrast, *Pythium* isolates were recovered from significantly ($P < 0.001$) more baits in assays with P_5ARP+B compared with P_5ARP+B+H medium (Fig. 3B). *Pythium* isolates were recovered in all months baiting assays were performed except one. This was not the case with *Phytophthora* isolates, with which no recovery occurred during the coldest periods of both years (Fig. 3A).

In surface baiting from the irrigation reservoir, *P. cryptogea* and *P. drechsleri* were the only *Phytophthora* spp. recovered on both media, except for a single recovery of an unidentified isolate on P_5ARP+B. Peaks of recovery of *P. drechsleri* occurred in August 2000 and July 2001 (Fig. 4). Peak recovery of *P. cryptogea* occurred later in both years and recovery of *P. cryptogea* continued 1 month after recovery of *P. drechsleri* had ceased (Fig. 4).

During quarterly baiting assays at 0, 1, and 1.5-m depths in the irrigation reservoir, mean percentage of recovery levels of both *Pythium* and *Phytophthora* isolates from leaf disk baits were not significantly different among the three depths in the irrigation reservoir on either medium (*data not shown*). However, a broader range of *Phytophthora* spp. was recovered in assays with P_5ARP+B+H medium at 1 and 1.5 m. Species recovered at these depths included *P. cryptogea, P. drechsleri, P. cactorum, P. capsici, P. citrophthora,* and *P. citricola*. However, the percentage of leaf disks from which *P. cactorum, P. capsici, P. citrophthora,* and *P. citricola* was recovered on P_5ARP+B+H medium during quarterly sampling at 1 and 1.5 m between May 2000 to November 2001 was low (i.e., 2, 9, 2, and 6%, respectively), as was frequency of recovery of these species (i.e., during single months).

DISCUSSION

Clearly, a diversity of *Phytophthora* spp. and a large number of propagules of *Pythium* spp. exist in the recycling water irrigation system at the nursery investigated in this study. In assays of nursery effluent, MacDonald et al. (19) had recovery levels of *Phytophthora* isolates that generally ranged from 0 to 400 CFU/liter, with relatively large fluctuations in propagule recovery at two of three nurseries that had implemented recycling water systems. The recovery of *Pythium* isolates outnumbered recovery of *Phytophthora* isolates in a manner comparable to the present work. Wilson et al. (34) found *Phytophthora* propagule levels up to 400 CFU/liter in assays of recycling water retention basins after implementation of irrigation water recycling. The range of recovery of *Phytophthora* isolates in the present work was somewhat lower, and levels of recovery fluctuated erratically in filtering assays from samples of chlorinated, nonchlorinated, and effluent water. However, the nursery investigated in this work had been treating irrigation water to kill waterborne pathogens. Relatively low levels of recovery may be an indication that the chlorine treatment reduced the numbers of viable propagules in irrigation water. Over the long term, effective water treatment may negate the possibility of populations of organisms reproducing exponentially in the recycling irrigation system. However, despite water treatment, some propagules of *Phytophthora* and *Pythium* spp. were able to survive in this irrigation water system and be spread through irrigation water. Any levels of these organisms in irrigation water are of concern to horticultural operations, because *Phytophthora* and *Pythium* spp. are associated with multicyclic disease and epidemics in favorable environments.

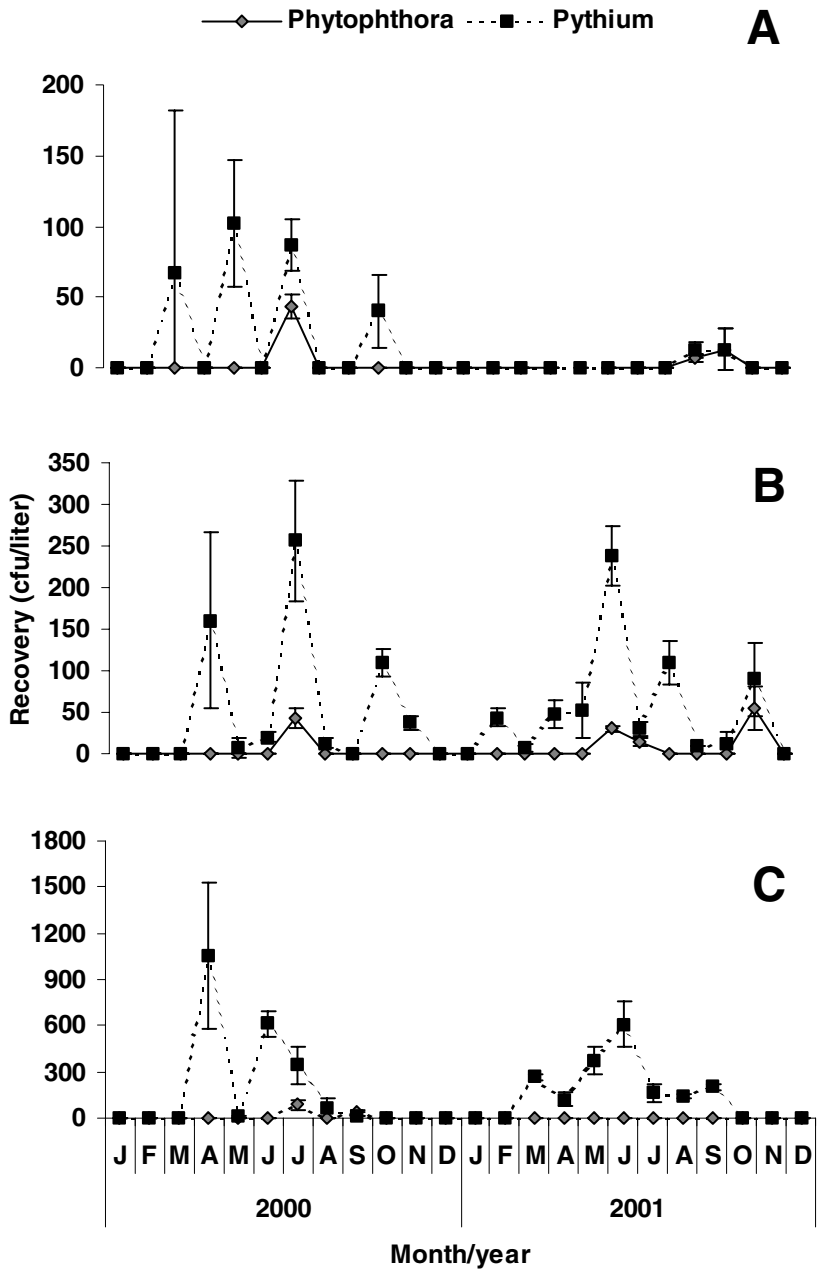

Fig. 2. *Phytophthora* and *Pythium* spp. recovered from **A,** chlorinated and **B,** nonchlorinated irrigation water and **C,** effluent water in filtering assays with P_5ARP agar plus benomyl (P_5ARP+B) medium. Each point represents the mean of three replicate assays. Error bars represent ± 1 standard deviation.

Phytophthora spp. recovered by filtering and baiting methods from the four water sources assayed include *P. capsici, P. citricola, P. citrophthora, P. cryptogea,* and *P. drechsleri*. *P. cactorum* and *P. nicotianae* were recovered only in baiting and filtering assays, respectively. However, *P. cactorum* was recovered in only a single instance and *P. nicotianae* only twice. Therefore, baiting and filtering methods may be similar in terms of detecting the range of species present in a water source. Recovery of *P. capsici* and *P. drechsleri* from nursery irrigation water has not been reported previously.

Although a relatively large diversity of *Phytophthora* spp. was identified, recovery of some species was quite limited. Specifically, during the 2-year filtering assays, *P. cactorum, P. capsici, P. citricola,* and *P. nicotianae* were recovered only one to four times and only between the months of greatest nursery activity from May through September. These species may be transient inhabitants of the nursery introduced by crops, and may be unable to survive and reproduce within the irrigation system. Alternatively, these species may not be recovered easily by the assay techniques employed in this work and may be recovered only when populations peak to threshold recovery levels.

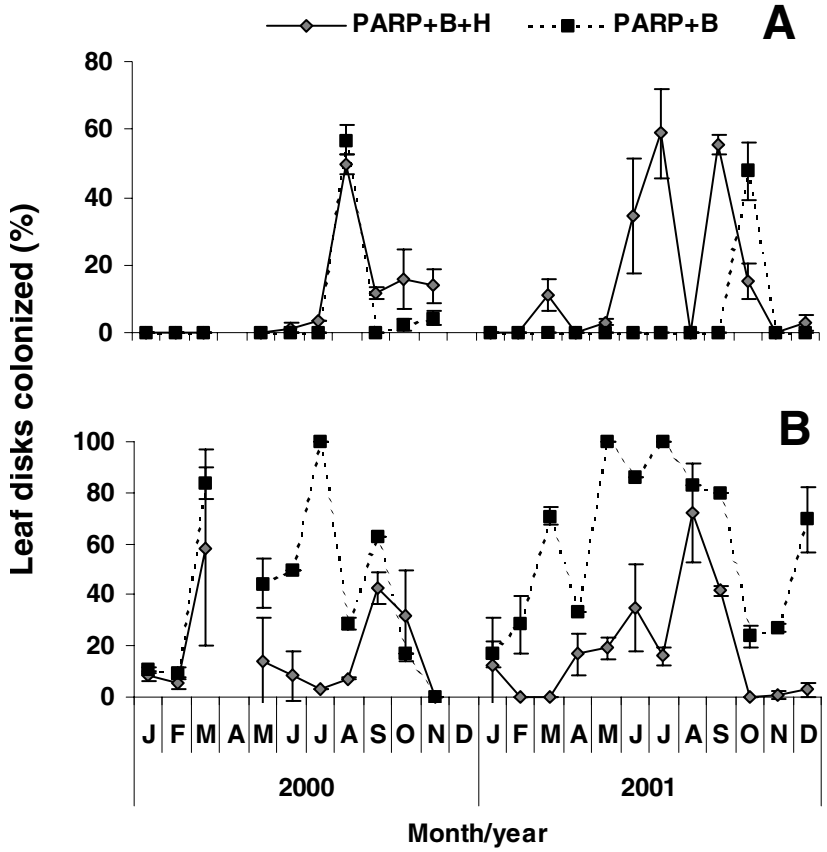

Fig. 3. Recovery of **A,** *Phytophthora* and **B,** *Pythium* spp. from rhododendron leaf bait disks placed on the surface of the irrigation reservoir. Leaf disks were plated on two different media: P_5ARP agar plus benomyl (P_5ARP+B) and P_5ARP+B amended with hymexazol (P_5ARP+B+H). Each point represents the mean of three replicate assays. Error bars represent ± 1 standard deviation. The mean percentage of leaf disks colonized was not significantly different between leaf disks plated on P_5ARP+B medium and leaf disks plated on P_5ARP+B+H medium ($P \leq 0.05$).

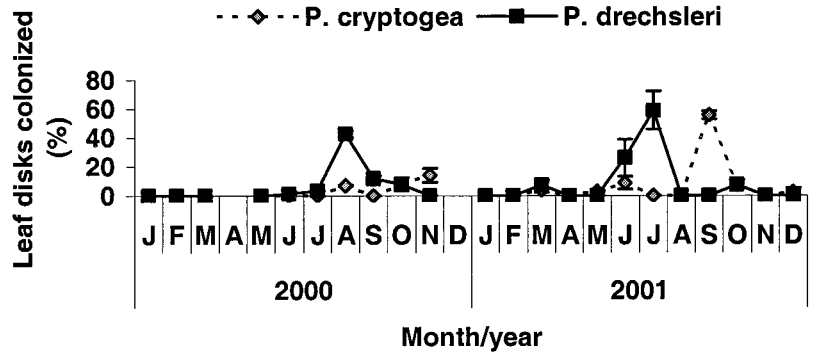

Fig. 4. Percentage of leaf disks colonized by *Phytophthora cryptogea* and *P. drechsleri* assays on P_5ARP agar plus benomyl amended with hymexazol. Baits were placed at the surface of the irrigation reservoir, which contains a mixture of fresh river water and recycled nursery effluent. Each point represents the mean of three replicate assays. Error bars represent ± 1 standard deviation.

P. drechsleri was the only species recovered from chlorinated irrigation water. It was recovered in filtering assays from both chlorinated and nonchlorinated irrigation water, whereas *P. citricola*, *P. citrophthora, P. cryptogea,* and *P. nicotianae* were recovered from both nonchlorinated irrigation water and nursery effluent. The greater diversity of species recovered from nonchlorinated water compared with chlorinated water does suggest that some species may be less sensitive to chlorination than other species, although sporadic recoveries make this impossible to ascertain.

P. drechsleri and *P. cryptogea* were the most frequently recovered species and their recovery occurred over the longest period, from early spring through fall, which suggests that these species are well adapted to the conditions and able to reproduce within the recycling irrigation system. However, recovery of these two species was primarily through rhododendron baits and recovery from effluent was extremely limited or lacking.

P. cryptogea and *P. drechsleri* were recovered more frequently from baits in the irrigation reservoir compared with recovery in filtering assays of nonchlorinated irrigation water, which originates from the same reservoir (Fig. 4). Shokes and McCarter (25) similarly observed that *Pythium* spp. were recovered by baiting even when no recovery occurred from the same source on selective media. Baiting allows germination and mycelial growth into plant tissue from the relatively delicate zoospore prior to transport or treatment in the laboratory. Additionally, motile zoospores possess positive chemotaxis (5) and may be attracted to baits. This would increase the probability of propagule recovery compared with sampling an arbitrary volume of water.

Baits have been considered very sensitive in assays for members of the family *Pythiaceae* (34), but also are problematic due to their unknown degree of selectivity for an organism (10). The competitive saprophytic ability of *P. cryptogea* has been reported by Bumbieris (4), who considered this species conspecific with *P. drechsleri.* Additionally, in water baiting assays, the higher frequency of recovery of *P. cryptogea* over other *Phytophthora* spp. prompted Taylor (30) to hypothesize that this species is parasitic on aquatic plants or terrestrial plants accessible by proximity to water, or that this species is saprophytic. Populations of saprobes or organisms with the ability to parasitize aquatic plants also could increase in water in the absence of a

terrestrial plant host. Reports of *P. cryptogea* in natural surface waters and the frequency of recovery of this species and the morphologically similar *P. drechsleri* also implicate river water, which is mixed with nursery effluent in the irrigation reservoir, as a possible source of these species. Further investigation of the lifecycles of these organisms may be warranted.

A greater diversity of *Phytophthora* spp. was recovered from baits placed below the water surface (i.e., at depths of 1 and 1.5 m), but no significant differences in overall recovery rates existed among the three baiting depths. These results indicate that setting baits between 1 to 1.5 m below the surface may be preferable to surface baiting in baiting assays for *Phytophthora* spp. Studies of aquatic fungi in lakes by Suzuki (28) showed that zoospores of *Pythium* spp. were equally distributed from the surface to the lake bottom; however, some zoospores of other aquatic fungi were aggregated at the depths near the bottom. The vertical distribution of zoospores of *Phytophthora* spp. in ponds, lakes, or irrigation reservoirs has not been investigated previously.

Although *P. citrophthora* was recovered from baits and nonchlorinated irrigation water, this species was primarily recovered in nursery effluent. This may indicate that propagules of this species are adapted to the relatively harsh conditions (e.g., rapid temperature fluctuations, ultraviolet light, and high salts) in effluent water or that populations in container plants are relatively high. The limited recovery of this species from other locations may indicate that this species is not as well adapted as *P. cryptogea* and *P. drechsleri* to a recycling irrigation system or that rhododendron leaf baits are not selective for this species. However, *P. citrophthora* was recovered over a relatively long period of the year (May through September).

Whether there is a positive correlation between levels of *Phytophthora* isolate recovery from nursery effluent and recovery levels in irrigation water cannot be proved in this study. Likewise, whether a positive correlation between species recovered from nursery effluent and corresponding species recovered from irrigation water exists also is uncertain. Factors contributing to this ambiguity include the discrete sampling time involved in this study. In a nursery situation, the number of crop species is quite large and the susceptibility of different crop species and ability to harbor potential pathogens is varied. Additionally, crop species are in dynamic flux in a nursery, unlike hosts in a natural setting or field plot. Pesticide applications and chlorine treatment represent other factors that add complexity to sampling and decrease the probability of firmly correlating factors in a nursery operation. Likewise, fluctuations in populations would be expected in irrigation water, depending on the length of time propagules are allowed to settle before effluent water is pumped into the irrigation reservoir, dilution of water in the reservoir by rainfall events, and sprays of chlorine to the irrigation reservoir to control algae bloom. For example, during these water assays, drought resulted in more time for propagules in the holding pond to settle out before reuse of collected effluent.

The two selective media performed differently in filtering- and baiting-based assays. Hymexazol-amended medium (e.g., P_5ARP+B+H) commonly is used to enhance recovery of *Phytophthora* spp. over faster-growing *Pythium* spp.; however, many *Phytophthora* spp. are sensitive to hymexazol (7). For example, Tay et al. (29) demonstrated that hymexazol decreases germination of zoospore cysts of *P. capsici*. The recovery of zoospores on the hymexazol-amended medium was most likely similarly reduced in this study. Therefore, *Phytophthora* isolate recovery may have been reduced through two mechanisms: (i) competition from faster growing *Pythium* spp. on non-hymexazol-amended media and (ii) decreases in viability of *Phytophthora* propagules on hymexazol-amended media. Although recovery of *Phytophthora* isolates was higher on media lacking hymexazol, it is probable that recovery would have been even higher if faster-growing *Pythium* spp. were suppressed. Therefore, serious limitations are apparent in assays for *Phytophthora* spp. that employ these two commonly used selective media. In baiting assays, a greater diversity of *Phytophthora* spp. was recovered with hymexazol-amended compared with nonamended medium, but significant differences in recovery levels of *Phytophthora* isolates were not demonstrated between the two media. Use of hymexazol-amended medium may enhance recovery of a greater range of *Phytophthora* spp. in baiting assays, but not necessarily in filtering assays.

Identification of *Phytophthora* spp. present in irrigation systems is the first step toward monitoring and managing these pathogens. Further determination of the relative importance of the species recovered, in terms of their pathogenicity and frequency of association with recycled irrigation water, will help to prioritize species for development of rapid detection tests. Compared to propagules of *Phytophthora*, *Pythium* propagules were much more abundant in irrigation water. The common occurrence of *Pythium* spp. in the water warrants their identification to species and an assessment of their importance as plant pathogens.

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