Applied and Environmental Microbiology, July 2009, p. 4307–4314 Vol. 75, No. 13
0099-2240/09/$08.00+0 doi:10.1128/AEM.00119-09

Zoosporic Tolerance to pH Stress and Its Implications for *Phytophthora* Species in Aquatic Ecosystems▽

Ping Kong,[1] Gary W. Moorman,[2] John D. Lea-Cox,[3] David S. Ross,[4] Patricia A. Richardson,[1] and Chuanxue Hong[1]*

Virginia Polytechnic Institute and State University, Hampton Roads Agricultural Research and Extension Center, Department of Plant Pathology, Physiology and Weed Science, Virginia Beach, Virginia 23455[1]; Pennsylvania State University, Department of Plant Pathology, University Park, Pennsylvania 16802[2]; University of Maryland, Department of Plant Science and Landscape Architecture, College Park, Maryland 20742[3]; and University of Maryland, Department of Environmental Science and Technology, College Park, Maryland 20742[4]

Received 18 January 2009/Accepted 4 May 2009

Phytophthora **species, a group of destructive plant pathogens, are commonly referred to as water molds, but little is known about their aquatic ecology. Here we show the effect of pH on zoospore survival of seven *Phytophthora* species commonly isolated from irrigation reservoirs and natural waterways and dissect zoospore survival strategy. Zoospores were incubated in a basal salt liquid medium at pH 3 to 11 for up to 7 days and then plated on a selective medium to determine their survival. The optimal pHs differed among *Phytophthora* species, with the optimal pH for *P. citricola* at pH 9, the optimal pH for *P. tropicalis* at pH 5, and the optimal pH for the five other species, *P. citrophthora*, *P. insolita*, *P. irrigata*, *P. megasperma*, and *P. nicotianae*, at pH 7. The greatest number of colonies was recovered from zoospores of all species plated immediately after being exposed to different levels of pH. At pH 5 to 11, the recovery rate decreased sharply ($P \leq 0.0472$) after 1-day exposure for five of the seven species. In contrast, no change occurred ($P \geq 0.1125$) in the recovery of any species even after a 7-day exposure at pH 3. Overall, *P. megasperma* and *P. citricola* survived longer at higher rates in a wider range of pHs than other species did. These results are generally applicable to field conditions as indicated by additional examination of *P. citrophthora* and *P. megasperma* in irrigation water at different levels of pH. These results challenge the notion that all *Phytophthora* species inhabit aquatic environments as water molds and have significant implications in the management of plant diseases resulting from waterborne microbial contamination.**

Phytophthora species, a group of oomycetes in the kingdom of Stramenopila and well-known plant pathogens, were first listed as "water molds" by Blackwell in 1944 (5), and this notion has since been generally accepted. These species are phylogenetically close to golden-brown algae, although morphologically and physiologically, they resemble fungi. Most algae are aquatic in nature. *Phytophthora* species produce flagellate zoospores as their primary dispersal structure (35–37, 39). Zoospores can travel in aquatic environments actively on their own locomotion and passively through water movement (12, 13, 41).

More than 20 species of *Phytophthora*, including *P. ramorum*, the sudden oak death pathogen, have been isolated from irrigation reservoirs and natural waterways (20–22, 30, 40, 43), and a number of previously unknown taxa also have been documented in aquatic environments (8, 24). These pathogens pose a threat to agricultural sustainability and natural ecosystems, as agriculture increasingly depends on recycled water for irrigation in light of rapidly spreading global water scarcity (19, 22). Recycling irrigation systems provide an efficient means of pathogen dissemination from a single point of infection to an entire farm and from a single farm to other farms sharing the same water resources (22, 24).

A search of science-based solutions to this crop health issue reveals a surprising lack of information on the aquatic ecology of *Phytophthora* species. For instance, hydrogen ion concentration (pH) is among the most important water quality parameters which influence sporangium production and germination (1–3, 6, 32, 34, 38), survival of thick-walled chlamydospores and oospores in the soil environment, and disease development (2, 4, 33, 44). However, the effect of pH on the survival of zoospores and growth of germlings in aquatic environments is not known. As motile zoospores lack cell walls and encysted spores or cysts have thin walls, they are presumably more vulnerable to pH stress than chlamydospores and oospores are. On the other hand, the pH level is likely to fluctuate more regularly and at a greater range in aquatic systems, such as irrigation reservoirs, than in soil systems. pH can change diurnally due to respiration of aquatic plants and seasonally due to rain, oxidation of sulfide-containing sediments through the production of sulfuric acid, algal blooms, and released bases or acids from residues of fertilizer and pesticides. Thus, zoospores and aquatic systems are more prone to the influence of wide pH changes than chlamydospores/oospores in soil systems are. The aim of this study was to determine the impact of pH on zoospore survival and understand the aquatic ecology of different *Phytophthora* species.

MATERIALS AND METHODS

Monitoring of pH in an irrigation reservoir. A 0.8-hectare (ha) irrigation reservoir at a 162-ha ornamental plant nursery in eastern Virginia was selected

* Corresponding author. Mailing address: Virginia Polytechnic Institute and State University, Hampton Roads Agricultural Research and Extension Center, 1444 Diamond Springs Road, Virginia Beach, VA 23455. Phone: (757) 363-3908. Fax: (757) 363-3950. E-mail: chhong2@vt.edu.

▽ Published ahead of print on 8 May 2009.

TABLE 1. Origin of *Phytophthora* isolates assessed in this study[a]

Phytophthora species	Isolate	Nursery[b]	Yr
P. citricola	43H1	A	2007
P. citrophthora	42E9	A	2007
P. insolita	37E3	B	2006
P. irrigata	42B9	B	2006
P. megasperma	42D2	C	2007
P. nicotianae	45H1	D	2007
P. tropicalis	7G9	E	2000

[a] All *Phytophthora* isolates were recovered from irrigation water.
[b] The individual nurseries from which these cultures were recovered were coded to protect the respective ornamental production enterprises.

to determine the range of water pH fluctuation over time. This reservoir was dug in 1998 and fed with rain and runoff water from sprinkler irrigation of an 8-ha production area where azalea, camellia, holly, juniper, maple, and rhododendron plants are grown in plastic containers on polyethylene sheets. The estimated volume of runoff water was approximately 34.9 m^3 day^{-1}.

Water pH was recorded hourly from 15 March 2006 to 10 June 2008 using a Hydrolab DS5X water quality multiprobe (Hach Environmental, Loveland, CO). The instrument was deployed near the middle of the reservoir (about 80 m from the runoff water entrance and 50 m from the outlet) and set to monitor water quality for water at a depth of 10 cm from the surface, where most *Phytophthora* species were recovered (9, 17). The recorded data were downloaded periodically into a surveyor (Hach Environmental) or directly to a laptop computer. At each downloading, pH was taken from additional depths at 50-cm intervals from the surface using the same instrument.

Test organisms. *Phytophthora citricola*, *Phytophthora citrophthora*, *Phytophthora insolita*, *Phytophthora irrigata*, *Phytophthora megasperma*, *Phytophthora nicotianae*, and *Phytophthora tropicalis*, were used in this study (Table 1). These species were selected because of their frequent recovery from recycling irrigation systems in the Mid-Atlantic region, including Virginia, Maryland, Pennsylvania, and North Carolina (9, 10, 20, 21) and from natural waterways in Virginia (C. Hong, unpublished data). These species were also selected because of their potential economic significance (14, 16, 20, 24, 25; USDA fungal databases at http://nt.ars-grin.gov/fungaldatabases/). Representative cultures of the species used were recovered from recycled irrigation water at ornamental plant nurseries in Virginia. Culture identities were confirmed by DNA fingerprints and morphology (16). The DNA fingerprinting technique is based on a single-strand conformation polymorphism analysis of rDNA internal transcribed spacer 1 (27).

Culture and zoospore production. Isolates were grown on clarified V8 agar (14) for 1 week. To induce sporangium production, mycelial plugs were removed and incubated in sterile soil water extract (14) for 8 to 16 h, then rinsed thoroughly with sterile distilled water (SDW), and placed under a fluorescent light overnight at room temperature (ca. 23°C). The plugs were then rinsed again with SDW and incubated in chilled SDW for 30 min to 2 h to facilitate zoospore release. Zoospore concentration was determined using a hemocytometer (Hausser Scientific, Horsham, PA).

Base media and pH adjustment. Hoagland's solution was used as a medium in most assays, as it represents a defined fertilizer and its contents are similar to those of slow-release fertilizers used in nurseries. The solution was prepared by dissolving 1.6 g of Hoagland's no. 2 basal salt mixture (Sigma-Aldrich Corp., St. Louis, MO) in 1 liter of deionized water. The solution was diluted to have a working concentration of 15% with an electrical conductivity (EC) of 430 μS cm^{-1}, which is in the range of measurements recorded from irrigation reservoirs. This working solution (base pH 5.3) was adjusted to pH 3 to 11 with NaOH or HCl to reflect the pH fluctuation range recorded in the reservoirs.

Irrigation water samples were collected from the same reservoir where water quality was monitored and used as the second test medium to verify the applicability of data obtained with the Hoagland's solution. To eliminate the interference from fauna, algae, and water molds, irrigation water samples were filtered through a 5-μm Durapore membrane filter (Millipore Corporation, Bedford, MA). The pH and EC of filtrate were measured by using a water quality checker (model U-10; Horiba, Ltd., Japan). The filtrates were used immediately or stored at 4°C for up to 2 weeks. Their base pH ranged from 7.0 to 9.0 and their EC ranging from 132 to 434 μS cm^{-1}; the base pH was adjusted to pH 3 to 11 as was done for Hoagland's solution. For refrigerated filtrates and Hoagland's solution, the pH was adjusted after they were equilibrated to room temperature.

Zoospore survival at different pHs. Sixty milliliters of the Hoagland's solution was dispensed into each of 100-ml autoclaved plastic bottles. A small volume of zoospore suspension was added to each bottle to make a final concentration of approximately 50 zoospores ml^{-1}. After gently swirling each bottle, a 1-ml aliquot was taken immediately (as a sample for day 0) and spread in a 90-mm petri dish containing a modified PARP-V8 agar (15, 23). The uncapped bottles were kept in a plastic container with water to minimize evaporation. The container was placed in a growth chamber at 23°C with a 10-h darkness/14-h light cycle. At approximately the same time of day, samples were taken from each bottle after 1-, 3-, 5-, and 7-day exposure and plated as described above. Sample plates were placed uncovered in a laminar flow cabinet (NuAire, Inc., Plymouth, MN) for about 30 min to allow evaporation before they were incubated in the dark at 23°C for 2 to 3 days. Emerging colonies were counted daily. The experiment was repeated at least twice with triplicate bottles for each pH level and exposure time combination in each run.

A comparative assessment on pH impact was performed between Hoagland's solution and filtrate of irrigation reservoir water in the same range of pH 3 to 11. This was done with the same isolate of *P. citrophthora* and *P. megasperma*, following the same procedures as described above. The experiment was repeated twice with three replicate samples per pH level and exposure time in each run.

Morphological changes in response to pH stress. Zoospores of *P. citrophthora* and *P. megasperma* were added to six-well plates with 5 ml of Hoagland's solution or filtrate of irrigation reservoir water per well to obtain a final concentration of 500 spores ml^{-1}. The plates were kept at 23°C. The morphological responses of zoospores to different levels of pH were examined 10 min and 2 and 24 h after onset of the experiment and thereafter daily, using an Olympus IX71 inverted microscope (Olympus, Center Valley, PA). Cysts and germinants were counted in three fields at a magnification of ×400 or ×100 for each well. Motile and lysing zoospores were noted when observed, but an absolute total count was not established. Relative counts (percentages) of cysts and germinated spores were computed by dividing each treatment by the greatest sum of these two counts across all pH levels and exposure times within the same species and test medium. The experiment was repeated at least once with three replicate wells for each pH treatment per run.

Statistical analyses. Water quality measurements recorded from the reservoir were summarized using Proc Freq of statistical analysis system (SAS) version 8 (SAS Institute, Inc., Cary, NC).

A relative recovery rate for all seven species was computed by dividing the colony counts of individual treatments by the highest average count for a given species and used as the measurement of zoospore survival at different pH and exposure time combinations. The relative recovery rates from repeated assays were pooled after homogeneity analyses and then subjected to Proc ANOVA of SAS to determine the impacts of pH and exposure time and their interactions on zoospore survival of the respective species. Duncan's multiple-range test was performed to determine the significance of differences in the relative recovery rate among pH levels at a given exposure time and among exposure times for a specific pH level as well as between the Hoagland's solution and irrigation water. Similarly, analysis of variance was performed to determine the difference in recovery between two test media, the Hoagland's solution versus filtrate of irrigation reservoir water.

RESULTS

Range of pH fluctuation in the irrigation reservoir. A total of 13,553 measurements were recorded over the 27-month monitoring period. The lowest surface water pH reading was 4.4, and the highest was 10.4. Most pH readings fell between pH 8 and 9 (33.3%), followed by pH 7 to 8 (29.5%) and pH 9 to 10 (25.7%). The reservoir water was alkaline for nearly 90% of the monitoring period. There was little difference in pH measurement across the water column which was approximately 1.5 m deep, depending on the time of year and weather conditions (data not shown).

Zoospore survival in Hoagland's solution at different pHs. Both pH level and exposure time exhibited significant impacts on zoospore survival of all species assessed in this study (Table 2). There also were significant interactions ($P < 0.0001$) between pH level and exposure time. As expected, the greatest recovery resulted from samples taken immediately (day 0) af-

TABLE 2. Relative zoospore survival of seven *Phytophthora* species as affected by pH in Hoagland's solution (15% strength)

Phytophthora species	Isolate	pH	Relative zoospore survival (%) exposed to the indicated pH for the following time (days) or *P* value[a]					*P* value[b]
			0	1	3	5	7	
P. citricola	43H1	3	22.4c	14.9ab	8.6bc	15.3a	5.4ab	0.1125
		5	**66.6**b	15.9ab	17.5a	8.3ab	3.3ab	<0.0001
		7	**77.7**b	0.3c	2.3bc	0.3b	0.0b	<0.0001
		9	**100**a	3.0bc	0.7c	0.3b	0.0b	<0.0001
		11	**42.3**c	18.4a	10.6ab	14.7a	7.3a	0.0014
		P value[c]	<0.0001	0.0160	0.0009	0.0007	0.0442	<0.0001
P. citrophthora	42E9	3	7.1c	5.7a	6.2a	4.0a	5.2a	0.9486
		5	**45.3**b	2.8a	0.7b	0.0a	1.4b	<0.0001
		7	**100**a	0.0a	0.0b	0.0a	0.0b	<0.0001
		9	**54.7**b	0.0a	0.0b	0.0a	0.7b	<0.0001
		11	**6.2**c	3.3a	0.0b	0.0a	0.0b	0.0472
		P value	<0.0001	0.3699	<0.0001	0.2245	0.0115	<0.0001
P. insolita	37E3	3	14.3c	9.5a	2.4ab	2.4b	2.4a	0.2532
		5	**56.1**b	5.4a	2.4ab	0.0b	0.0b	<0.0001
		7	**100**a	2.4a	0.0b	0.0b	0.0b	<0.0001
		9	**74.4**ab	6.7a	6.7ab	0.0b	0.0b	<0.0001
		11	41.4bc	22.4a	16.7a	24.8a	4.8a	0.3527
		P value	0.0009	0.2322	0.1342	0.0035	0.2341	<0.0001
P. irrigata	42B9	3	10.6c	0.0a	7.1a	9.5a	3.5a	0.2658
		5	**49.9**b	4.7a	4.8a	4.8a	0.0a	<0.0001
		7	**100**a	3.5a	0.0a	0.0a	0.0a	<0.0001
		9	**59.6**b	3.4a	0.0a	2.4a	0.0a	<0.0001
		11	**17.6**c	2.4a	0.0a	0.0a	0.0a	0.0001
		P value	<0.0001	0.6096	0.1762	0.2170	0.1103	<0.0001
P. megasperma	42D2	3	35.2c	20.0ab	18.9ab	15.2a	10.5b	0.4905
		5	56.7bc	40.6a	54.4a	32.1a	47.5a	0.5838
		7	**100**a	41.5a	40.8ab	32.6a	12.8b	0.0047
		9	**77.8**ab	13.9ab	15.2b	17.5a	17.5b	<0.0001
		11	**28.4**c	4.7b	10.5b	1.2a	7.0b	0.0024
		P value	0.0004	0.0222	0.0629	0.2251	0.0591	0.0790
P. nicotianae	45H1	3	7.9c	4.7a	4.7a	3.1a	3.1a	0.7209
		5	**53.3**b	4.7a	1.6a	0.0a	0.0a	0.0007
		7	**100**a	3.2a	1.6a	0.0a	0.0a	<0.0001
		9	**41.3**b	7.9a	0.0a	0.0a	0.0a	<0.0001
		11	**12.6c**	4.7a	3.1a	3.1a	0.0a	0.0037
		P value	<0.0001	0.7590	0.5168	0.1462	0.0681	<0.0001
P. tropicalis	7G9	3	19.6c	9.0a	5.0a	5.8a	7.8a	0.1422
		5	**100**a	4.3a	2.1ab	0.2b	0.2b	<0.0001
		7	**63.0**b	6.4a	0.2b	1.5b	0.0b	<0.0001
		9	**74.9**b	3.5a	2.1ab	0.0b	0.0b	<0.0001
		11	**33.5**c	1.9a	0.0b	0.0b	0.0b	<0.0001
		P value	<0.0001	0.2230	0.0948	0.0162	0.0066	<0.0001

[a] Zoospore survival values and *P* values for the interactions between pH and exposure time according to analysis of variance. Values followed by the same letter within each column and species did not differ according to Duncan's multiple-range test at a *P* value of 0.05. Value(s) that differed from the other values within each row according to Duncan's multiple-range test at a *P* value of 0.05 are shown in boldface type.

[b] Significance (*P* values) of differences (zoospore survival values) among exposure times at a specified pH.

[c] Significance (*P* values) of differences (zoospore survival values) among pH levels at a given exposure time.

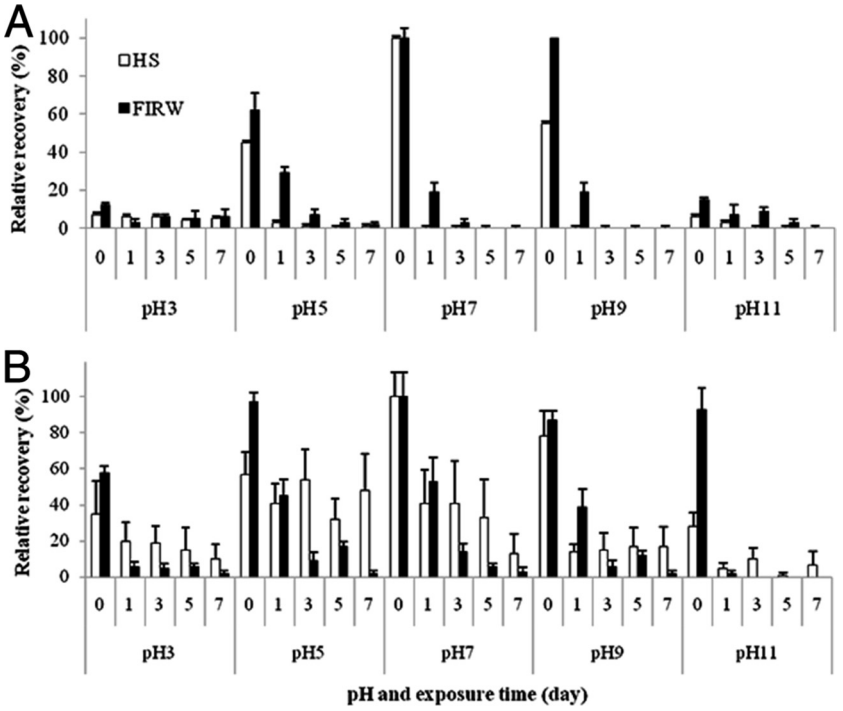

FIG. 1. Comparative zoospore recovery of *Phytophthora citrophthora* (A) and *P. megasperma* (B) after incubating in Hoagland's solution (HS) and filtrate of irrigation reservoir water (FIRW) at different pH levels for up to 7 days. The values are the means plus standard errors (error bars) for three tests.

ter zoospores were added to Hoagland's solution at different pH levels (Table 2). The optimal pH differed with *Phytophthora* species: *P. citricola* at pH 9, *P. tropicalis* at pH 5, and at pH 7 for the five other species, *P. citrophthora*, *P. insolita*, *P. irrigata*, *P. megasperma*, and *P. nicotianae*. The recovery rate of individual species decreased substantially ($P \leq 0.0009$) as the solution pH moved away from their optima (Table 2).

Two general patterns of survival were observed in association with extended periods of exposure to different pH treatments (Table 2). At pH 5 to 11, the recovery rate of all species declined sharply ($P \leq 0.0472$) as the exposure time was extended from day 0 to 1, but it did not decline further when the exposure time was extended from day 1 to 7. The only two exceptions were *P. insolita* at pH 11 ($P = 0.3527$) and *P. megasperma* at pH 5 ($P = 0.5838$). At pH 3, there was little or no change in the relative recovery of all species among the five exposure periods.

The recovery rate differed greatly among the species at different pH levels and exposure times (Table 2). *P. megasperma* was recovered after 7-day exposure at all pH levels at rates of 7.0 to 47.5%. This species survived at the highest rates in the broadest range of pHs for the longest exposure period (7 days) among the seven species assessed in this study. The next species in rank was *P. citricola*, which was recovered after 7-day exposure at pH 3, 5, and 11, but not at pH 7 and 9. These two species were followed by *P. insolita*, *P. tropicalis*, *P. citrophthora*, *P. irrigata*, and *P. nicotianae*. *P. tropicalis* appeared to favor acidic conditions, since the zoospores were rarely recovered at pH 7 or higher, while *P. insolita* survived well under highly alkaline conditions at pH 11.

Comparative survival in irrigation water and Hoagland's solution. *Phytophthora citrophthora* survived longer and at higher rates in the filtrate of irrigation water than in the Hoagland's solution, although the general recovery patterns from these two media were similar (Fig. 1A). The greatest recovery was at day 0 when zoospores were incubated in the filtrate. Similarly, the recovery rate did not change with extended exposure at pH 3. On the other hand, this species was recovered at three exposure periods at pH 7, two at pH 9, and 4 at pH 11 in the filtrate as opposed to only 1 at pH 7 and 9 and 2 at pH 11 in the Hoagland's solution. Likewise, zoospores were recovered at higher rates from all exposure periods in the filtrate than in the nutrient solution at pH 5.

As in the Hoagland's solution, *P. megasperma* in the filtrate survived at a wide range of pHs for almost all test periods (Fig. 1B). The relative recovery rates of this species were greater than those in the Hoagland's solution at some levels of pH for the first two exposure times (days 0 and 1). However, this species survived at discernibly lower rates in the filtrate than in the Hoagland's solution if zoospores were treated longer (days 3, 5, and 7) at all pH levels.

Morphological changes in response to pH stress. Morphological changes of zoospores under pH stress are illustrated with *P. citrophthora* in the Hoagland's solution (Fig. 2). Swimming zoospores were observed at pH 5 for up to 24 h and at pH 7 for 4 h in the Hoagland's solutions. In contrast, they were observed for a few minutes at pH 3, 9, and 11. Unfavorable ambient pH prompted motile zoospores to encyst or lyse. Some cysts also lysed. For instance, at pH 3, most zoospores encysted instantly, and a few lysed (Fig. 2A). Encysted zoo-

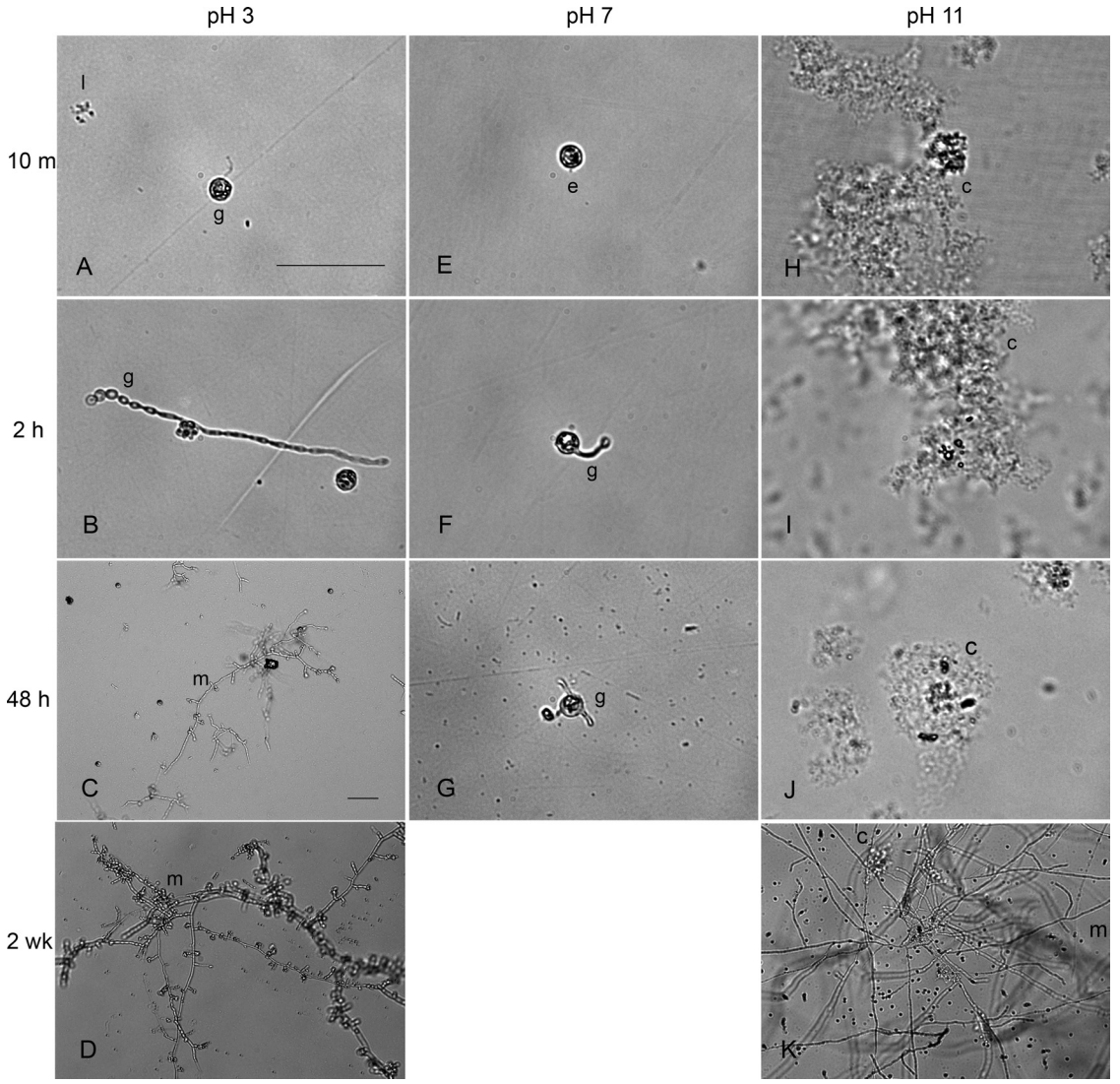

FIG. 2. Micrographs illustrating the impact of pH on zoospores and germlings of *Phytophthora citrophthora* in 15% Hoagland's solution over a 2-week period. The effects of pH 3, 7 and 11 for different lengths of time (from 10 minutes [10 m] to 2 weeks) are shown. Abbreviations: l, lysed zoospore; g, germling; m, mycelium; e, encysted zoospore; c, cytoplasmic mass. Bar, 50 μm. Panels C, D, and K were taken at a magnification of ×100, and the other panels were taken at a magnification of ×400.

spores at this pH tended to germinate rapidly (Fig. 2B), although the resultant germlings grew abnormally (Fig. 2C and D). At pH 7, only a small portion of zoospores lost motility and became encysted within 10 min (Fig. 2E). Encysted zoospores germinated (Fig. 2F), but the germinants grew slowly (Fig. 2G). Most zoospores and germlings lysed after a 3-day exposure at pH 7. Neither mycelial growth nor zoospores were observed at this pH after 1- or 2-week exposure. At pH 11, a large portion of zoospores lysed instantly (Fig. 2H, I, and J) but some cytoplasmic masses initiated new growth at day 3 and extensive mycelium was observed after 2-week exposure (Fig. 2K).

For *P. citrophthora* in the Hoagland's solution, the relative percentage of cysts increased with increasing acidity (Fig. 3). The greatest rate and rapidity of zoospore encystment occurred at pH 3, as did cyst germination. Encystment and germination peaked at 48 h. Subsequently, encystment slowed and cyst lysis increased, resulting in a declining percentage of cysts and germinated zoospores with increasing exposure time. Approximately 20% of cysts germinated at 48 and 72 h. Most zoospores lysed at pH 9, and fewer cysts germinated at this pH. Only about 10% of zoospores became encysted immediately after addition to the solution, and the encystment rate decreased with increasing time of exposure. At pH 11, the majority of zoospores lysed instantly. However, most of the cytoplasmic masses remained, and some produced substantial mycelia.

In comparison, zoospores of *P. citrophthora* in the filtrate of irrigation reservoir water remained motile for longer periods of time than those in Hoagland's solution. They swam for 48 h at pH 7 and 9. As in the Hoagland's solution, the highest encystment was observed at pH 3, but few cysts germinated (Fig. 4A).

Compared to *P. citrophthora*, *P. megasperma* in the filtrate responded to pH stress differently, resulting in greater rates of encystment and germination across all treatments (Fig. 4B). At pH 3, more zoospores became encysted, which occurred al-

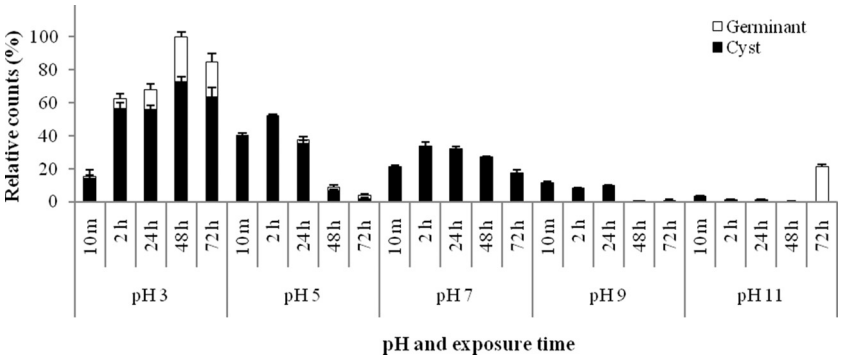

FIG. 3. Relative percentages of encysted zoospores and germinated cysts of *Phytophthora citrophthora* in 15% Hoagland's solution at different pH levels and exposure times (from 10 minutes [10 m] to 72 h). Each bar shows the average plus standard error (error bar) of 18 counts in three replicate wells from two experiments.

most instantly; fewer zoospores and cysts lysed at every examination from 10 min to 72 h; some cysts germinated and developed into chain-like mycelia after 1 week. At higher levels of pH (5 to 11), a greater rate of encystment occurred, and substantial number of cysts germinated across all exposure times with one exception. Fewer zoospores, cysts, and germinated cysts lysed, which occurred mostly at pH 11. The higher survival of zoospores, cysts, and germinants all contributed to the greater recovery rate of this species (Fig. 1).

DISCUSSION

Zoospores function as both dispersal and infective propagules in the life cycle of *Phytophthora* species (14). As zoospores are the principal infective propagule, zoospore behavior has been a focal point of numerous previous studies (12, 26, 41). In those studies, it was clear that pH can alter zoospore motility (18, 28). However, it was not known what range of pH fluctuation *Phytophthora* zoospores may encounter in an agricultural aquatic ecosystem and how zoospores survive under such fluctuating pH environments. Our study reveals that hydrogen ion concentration in irrigation reservoirs fluctuates dramatically (10^6 times from its lowest to highest points) and is in the alkaline condition for most of the time during the growing season. Such a range of pH fluctuation is a highly significant factor limiting the survival of zoospores of *Phytophthora* species in the aquatic environment. These findings have important implications in understanding the aquatic ecology of these pathogens and water decontamination technology innovation.

The results of our study challenge the general notion that

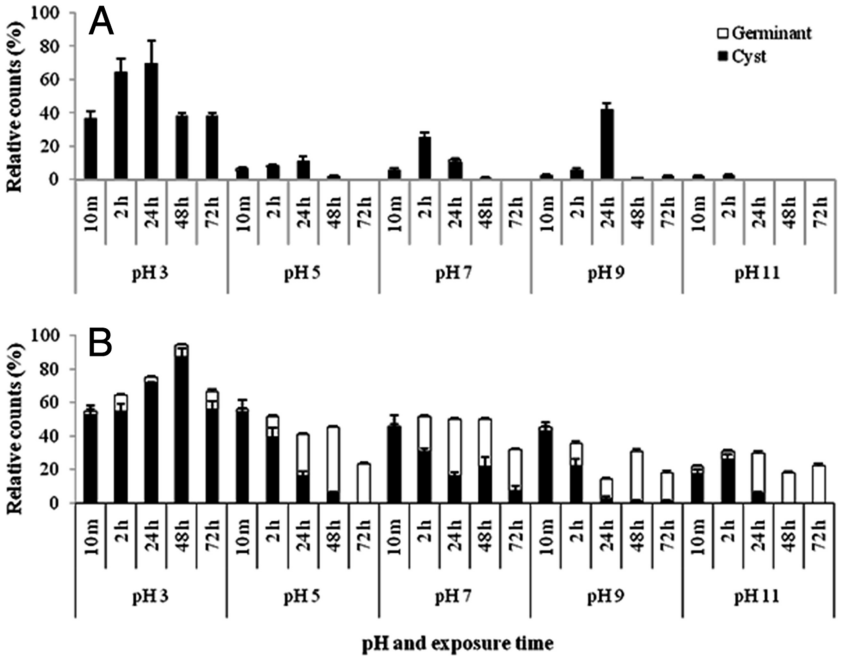

FIG. 4. Relative percentages of encysted zoospores and germinated cysts of *Phytophthora citrophthora* (A) and *P. megasperma* (B) in filtrate of irrigation reservoir water at different pH levels and exposure times (from 10 minutes [10 m] to 72 h). Each bar shows the average plus standard error (error bar) of 18 counts in three replicate wells from two experiments.

Phytophthora species are water molds, at least in terms of zoospore activity. Some *Phytophthora* species, such as *P. citrophthora*, may not live as long in aquatic environments as initially perceived. This is particularly true for agricultural irrigation reservoirs where surface runoff water is collected and reused for irrigation. Such aquatic ecosystems continuously receive nutrients through runoff water, which results in an increased degree of eutrophication and fluctuation in water quality, including pH. Zoospores account for at least 95% of propagules recovered in aquatic ecosystems (11, 29, 35, 39). However, for five of the seven species assessed in this study, most zoospores did not survive beyond 24 h in Hoagland's solution, and their mortality increased instantly with increasing pH stress (Table 2). This observation is supported by the results of tests with *P. citrophthora* (representing pH-sensitive species) and *P. megasperma* (pH stress-tolerant species) in filtrate of irrigation reservoir water (Fig. 1).

Zoosporic pH stress tolerance in an aquatic environment varies greatly with species. The seven *Phytophthora* species assessed in this study had three optimal pHs: pH 7 (neutral) for *P. citrophthora*, *P. insolita*, *P. irrigata*, *P. megasperma*, and *P. nicotianae*, pH 5 (acidic) for *P. tropicalis*, and pH 9 (alkaline) for *P. citricola* (Table 2). Overall, *P. megasperma* survived the longest (throughout the 7-day test period) at the highest rates in the widest range of pHs, followed by *P. citricola* (Table 2). These results support the findings of previous investigations where these two species were most frequently recovered from water sampling at different ornamental plant nurseries throughout the Commonwealth of Virginia (9, 10, 17) and in other states and countries (22). It is interesting that all species survived at the extreme pH of 3 and some at pH 11 as well (Table 2). These extreme pH conditions induced zoospore encystment and the formation of some deformed structures, although most zoospores or cysts lysed at pH 11 as illustrated by *P. citrophthora*. Cysts are less vulnerable to lysis than motile zoospores are; the surviving structures are tolerant, and some can even initiate new growth. Surviving cysts of *P. citrophthora* germinated as early as 2 h after exposure at pH 3, and the cytoplasmic masses resulting from lysed cysts of *P. citrophthora* at pH 11 resumed growth after 72 h; both had considerable growth after 2-week exposure (Fig. 2). Cysts are capable of repeated emergence (42); thus, prompt encystment presents an advantage for zoospores as a dispersal and infective structure. This tolerance to extreme pH of zoospores may indicate another survival strategy of *Phytophthora* species in the environment. On the other hand, zoospores under their optimal or "less detrimental" pH conditions, such as pH 5 and 7, are short-lived (Table 2), although they tend to remain motile longer than those at other pH levels. *P. megasperma* stands out as the most adaptive to aquatic environments as shown for survival at all levels of pH and exposure periods tested (Table 2). This is attributed to the sustained greater survival of its zoospores, cysts, and germinants across all levels of pH assessed (Fig. 4B).

This study is one step in understanding the aquatic ecology of *Phytophthora* species. Our results clearly indicate that not all species are equally adapted to aquatic environments as water molds, at least in terms of zoospore activity. Among the seven species assessed in this study, *P. megasperma* has the widest pH tolerance, followed by *P. citricola* and then the other five species. The number of recognized species within the genus *Phytophthora* has expanded in recent years and that number is approaching 100 (16). Thus, zoosporic pH stress tolerance of the vast majority of *Phytophthora* species in aquatic environments is yet to be investigated. Also, the hydrogen ion concentration is only one of numerous water quality parameters. Other water quality parameters could have profound impacts on survival of *Phytophthora* species in aquatic ecosystems and warrant investigation. Thus, much is yet to be learned about how *Phytophthora* species may survive in different aquatic environments. In addition, the results of the present study contrast with those observed in soil systems where *Phytophthora* species are considered tolerant to a wide range of pH from 3.8 to 9.0 (44) and increasing pH generally favored growth (2, 33, 44). This contrast also highlights the urgent need for intensifying studies of the aquatic ecology of this group of important plant pathogens.

The present study also has several applications. Zoospore mortality in Hoagland's solution within the first day of testing is indicative of the pH stress tolerance of *Phytophthora* species in aquatic environments. Thus, future studies of pH stress tolerance of other important *Phytophthora* species, such as *P. ramorum* (31) and *P. kernoviae* (7), may concentrate on the first day. The assay and treatment times also may be adapted to assess the impact of other water quality parameters on survival of zoospores in aquatic ecosystems, and this possibility certainly deserves investigation. More importantly, our results are essential to assess the dissemination risk of individual *Phytophthora* species through irrigation water and devise novel water treatment technologies.

ACKNOWLEDGMENT

This research was supported in part by a grant from the USDA Cooperative State Research, Education and Extension Service Risk Avoidance and Mitigation Program (2005-51101-02337).

REFERENCES

1. **Allen, D. J., and S. S. Nandra.** 1975. Effects of pH and calcium concentration of sporulation of *Phytophthora* isolates from agave. Plant Dis. Rep. **59:**555–558.
2. **Andrivon, D.** 1994. Fate of *Phytophthora infestans* in a suppressive soil in relation to pH. Soil Biol. Biochem. **26:**953–956.
3. **Benson, D. M.** 1984. Influence of pine bark, matric potential, and pH on sporangium production by *Phytophthora cinnamomi*. Phytopathology **74:**1359–1363.
4. **Bingham, F. T., and G. A. Zentmyer.** 1954. Relation of hydrogen-ion concentration in nutrient solution to *Phytophthora* root rot of avocado seedlings. Phytopathology **44:**611–614.
5. **Blackwell, E.** 1944. Species of *Phytophthora* as water moulds. Nature **153:**496.
6. **Blaker, N. S., and J. D. MacDonald.** 1983. Influence of container medium pH on sporangium formation, zoospore release, and infection of rhododendron by *Phytophthora cinnamomi*. Plant Dis. **67:**259–263.
7. **Brasier, C. M., P. A. Beales, S. A. Kirk, S. Denman, and J. Rose.** 2005. *Phytophthora kernoviae* sp. nov., an invasive pathogen causing bleeding stem lesions on forest trees and foliar necrosis of ornamentals in the UK. Mycol. Res. **109:**853–859.
8. **Brasier, C. M., D. E. L. Cooke, J. M. Duncan, and E. M. Hansen.** 2003. Multiple new phenotypic taxa from trees and riparian ecosystems in *Phytophthora gonapodyides-P. megasperma* ITS clade 6, which tend to be high-temperature tolerant and either inbreeding or sterile. Mycol. Res. **107:**277–290.
9. **Bush, E. A., C. X. Hong, and E. L. Stromberg.** 2003. Fluctuations of *Phytophthora* and *Pythium* spp. in components of a recycling irrigation system. Plant Dis. **87:**1500–1506.
10. **Bush, E. A., E. L. Stromberg, C. X. Hong, P. A. Richardson, and P. Kong.** 2006. Illustration of key morphological characteristics of *Phytophthora* species identified in Virginia nursery irrigation water. Plant Health Prog. doi: 10.1094/PHP-2006-0621-01-RS.
11. **Charlton, N. D., and S. L. von Broembsen.** 2000. Survival, settling, and

lateral dispersal of encysted zoospores of *Phytophthora* spp. in captured irrigation runoff. Phytopathology **90:**S13.
12. **Deacon, J. W., and S. P. Donaldson.** 1993. Molecular recognition in the homing responses of zoosporic fungi, with special reference to *Pythium* and *Phytophthora*. Mycol. Res. **97:**1153–1171.
13. **Duniway, J. M.** 1979. Water relation of water molds. Annu. Rev. Phytopathol. **17:**431–460.
14. **Erwin, D. C., and O. K. Ribeiro.** 1996. Phytophthora diseases worldwide. APS Press, St. Paul, MN.
15. **Ferguson, A. J., and S. N. Jeffers.** 1999. Detecting multiple species of *Phytophthora* in container mixes from ornamental crop nurseries. Plant Dis. **83:**1129–1136.
16. **Gallegly, M. E., and C. X. Hong.** 2008. *Phytophthora*: identifying species by morphology and DNA fingerprints. APS Press, St. Paul, MN.
17. **Ghimire, S. R., P. A. Richardson, G. W. Moorman, J. D. Lea-Cox, D. S. Ross, and C. X. Hong.** 2009. An *in-situ* baiting bioassay for detecting *Phytophthora* species in irrigation runoff containment basins. Plant Pathol. **58:**577–583. doi:10.1111/j.1365-3059.2008.02016.x.
18. **Ho, H. H., and C. J. Hickman.** 1967. Asexual reproduction and behavior of zoospores of *Phytophthora megasperma* var. *sojae*. Can. J. Bot. **45:**1963–1981.
19. **Hong, C. X., E. A. Bush, P. A. Richardson, and E. L. Stromberg.** 2001. The major deterrent to recycling irrigation water in nursery and greenhouse operations despite lack of alternatives for limiting nonpoint source pollution, p. 72–77. Proceedings of the Virginia Water Research Symposium 2001. Protecting our water resources for the next generation. Where do we go from there? Virginia Water Resources Research Center, Virgina Polytechnic Institute and State University, Blacksburg, VA.
20. **Hong, C. X., M. E. Gallegly, P. A. Richardson, P. Kong, and G. W. Moorman.** 2008. *Phytophthora irrigata*, a new species isolated from irrigation reservoirs and rivers in eastern United States of America. FEMS Microbiol. Lett. **285:**203–211.
21. **Hong, C. X., M. E. Gallegly, P. A. Richardson, P. Kong, G. W. Moorman, J. D. Lea-Cox, and D. S. Ross.** 2008. *Phytophthora irrigata* and *Phytophthora hydropathica*, two new species from irrigation water at ornamental plant nurseries. Phytopathology **100:**S68.
22. **Hong, C. X., and G. W. Moorman.** 2005. Plant pathogens in irrigation water: challenges and opportunities. Crit. Rev. Plant Sci. **24:**189–208.
23. **Hong, C. X., P. A. Richardson, and P. Kong.** 2002. Comparison of membrane filters as a tool for isolating pythiaceous species from irrigation water. Phytopathology **92:**610–616.
24. **Hong, C. X., P. A. Richardson, and P. Kong.** 2008. Pathogenicity to ornamental plants of some existing species and new taxa of *Phytophthora* from irrigation water. Plant Dis. **92:**1201–1207.
25. **Hong, C. X., P. A. Richardson, P. Kong, S. N. Jeffers, and S. W. Oak.** 2006. *Phytophthora tropicalis* isolated from diseased leaves of *Pieris japonica* and *Rhododendron catawbiense* and found in irrigation water and soil in Virginia. Plant Dis. **90:**525.
26. **Judelson, H. S., and F. A. Blanco.** 2005. The spores of *Phytophthora*: weapons of the plant destroyer. Nat. Rev. Microbiol. **3:**47–58.
27. **Kong, P., C. X. Hong, P. A. Richardson, and M. E. Gallegly.** 2003. Single-strand-conformation polymorphism of ribosomal DNA for rapid species differentiation in genus *Phytophthora*. Fungal Genet. Biol. **39:**238–249.
28. **Morris, B. M., B. Reid, and N. A. R. Gow.** 1995. Tactic response of zoospores of the fungus *Phytophthora palmivora* to solutions of different pH in relation to plant infection. Microbiology **141:**1231–1237.
29. **Pittis, J. E., and J. Colhoun.** 1984. Isolation and identification of pythiaceous fungi from irrigation water and their pathogenicity to *Antirrhinum*, tomato and *Chamaecyparis lawsoniana*. Phytopathol. Z. **110:**301–318.
30. **Reeser, P. W., E. M. Hansen, and W. Sutton.** 2007. *Phytophthora siskiyouensis*, a new species from soil, water, myrtlewood (*Umbellularia californica*) and tanoak (*Lithocarpus desifloorus*) in southwestern Oregon. Mycologia **99:**639–643.
31. **Rizzo, D. M., M. Garbelotto, and E. A. Hansen.** 2005. *Phytophthora ramorum*: integrative research and management of an emerging pathogen in California and Oregon forests. Annu. Rev. Phytopathol. **43:**309–335.
32. **Sato, N.** 1994. Effect of some inorganic salts and hydrogen ion concentration on indirect germination of the sporangia of *Phytophthora infestans*. Ann. Phytopathol. Soc. Jpn. **60:**441–447.
33. **Schmitthenner, A. F., and C. H. Canaday.** 1983. Role of chemical factors in development of *Phytophthora* diseases, p. 189–196. *In* D. C. Erwin, S. Bartnicki-Garcia, and P. H. Tsao (ed.), Phytophthora: its biology, taxonomy, ecology, and pathology. APS Press, St. Paul, MN.
34. **Simpfendorfer, S., T. J. Harden, and G. M. Murray.** 2001. Effect of temperature and pH on the growth and sporulation of *Phytophthora clandestina*. Aust. Plant Pathol. **30:**1–5.
35. **Stanghellini, M. E., D. H. Kim, S. L. Rasmussen, and P. A. Rorabaugh.** 1996. Control of root rot of peppers caused by *Phytophthora capsici* with a nonionic surfactant. Plant Dis. **80:**1113–1116.
36. **Stanghellini, M. E., and R. M. Miller.** 1997. Biosurfactants: their identity and potential in the biological control of zoosporic plant pathogens. Plant Dis. **81:**4–12.
37. **Stanghellini, M. E., and S. L. Rasmussen.** 1994. Hydroponics: a solution for zoosporic pathogens. Plant Dis. **78:**1129–1138.
38. **Suzaki, E., T. Suzaki, S. L. Jackson, and A. R. Hardham.** 1996. Changes in intracellular pH during zoosporogenesis in *Phytophthora cinnamomi*. Protoplasma **191:**79–83.
39. **Thomson, S. V., and R. M. Allen.** 1974. Occurrence of *Phytophthora* species and other potential plant pathogens in recycled irrigation water. Plant Dis. Rep. **58:**945–949.
40. **Tjosvold, S. A., D. L. Chambers, S. T. Koike, and S. R. Mori.** 2008. Disease on nursery stock as affected by environmental factors and seasonal inoculum levels of *Phytophthora ramorum* in stream water used for irrigation. Plant Dis. **92:**1566–1573.
41. **Tyler, B. M.** 2002. Molecular basis of recognition between *Phytophthora* pathogens and their hosts. Annu. Rev. Phytopathol. **40:**137–167.
42. **von Broembsen, S. L., and N. D. Charlton.** 2000. Repeated zoospore emergence by several *Phytophthora* spp. found in irrigation water. Phytopathology **90:**S81.
43. **Werres, S., S. Wagner, T. Brand, K. Kaminski, and D. Seipp.** 2007. Survival of *Phytophthora ramorum* in recirculating irrigation water and subsequent infection of *Rhododendron* and *Viburnum*. Plant Dis. **91:**1034–1044.
44. **Weste, G.** 1983. Population dynamics and survival of *Phytophthora*, p. 237–258. *In* D. C. Erwin, S. Bartnicki-Garcia, and P. H. Tsao (ed.), Phytophthora: its biology, taxonomy, ecology and pathology. APS Press, St. Paul, MN.