EPIDEMIOLOGY AND MANAGEMENT OF *PHYTOPHTHORA* SPP. IN RUNOFF FROM ORNAMENTAL NURSERIES USING RECYCLING IRRIGATION SYSTEMS

By

NIKKI DE-ROI CHARLTON

Bachelor of Science

Oklahoma State University

Stillwater, Oklahoma

1999

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 2001

EPIDEMIOLOGY AND MANAGEMENT OF *PHYTOPHTHORA* SPP.

IN RUNOFF FROM ORNAMENTAL NURSERIES USING

RECYCLING IRRIGATION SYSTEMS

Thesis Approved:

Thesis Adviser

Dean of the Graduate College

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to my major advisor, Dr. Sharon von Broembsen for her intelligent supervision, guidance, inspiration, and friendship. My sincere appreciation extends to my other committee members Dr. Michael Smolen and Dr. Larry Littlefield, whose guidance, assistance, and encouragement also were invaluable. I would like to thank the Horticultural Research Institute for providing me with this research opportunity and their generous financial support. Acknowledgment is also due to the Department of Entomology and Plant Pathology for financial support over the past two years.

I would like to thank Brad Cost for his assistance with constructing experimental equipment as well as his contribution to the artwork. I am grateful to Mark Trent and Matt West for their technical assistance with the field experiments. Finally I would like to extend special appreciation to my parents for their continued support and encouragement.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

LIST OF FIGURES

Figure Page

CHAPTER I

LITERATURE REVIEW

Capture and recycle technology as a pollution prevention strategy. A major environmental concern regarding ornamental nurseries is the impact of nutrients and pesticides in irrigation runoff. Nitrogen, phosphorus, and other trace elements are the nutrient pollutants found in recycled irrigation water (EPA, 1992). Federal, state, and local regulations have been implemented to control the management of irrigation wastewater, to prevent nutrient and pesticide contamination of water resources (MacDonald et al, 1994b). Surface runoff water may contaminate streams and lakes, and excess irrigation water may percolate into groundwater depending on the site. Production agriculture is labeled as one of the primary contributors to the pollution of groundwater (Wright, 1988). Nurseries are considered to be an agricultural non-point source of pollution (Fain et al, 1998), however they may be considered point sources in the U.S. in the near future (MacDonald et al, 1994a). Capturing nursery runoff and reusing it for irrigation is one way to assure that fertilizer and pesticides in runoff do not contaminate surface waters. Implementing a capture and recycle system in container nurseries prevents pollution, reduces water costs and provides storm water control.

The adoption of capture and recycle technology as a pollution prevention strategy has been hindered by the potential for increased disease and costly disinfestations caused by recycling plant pathogens. Irrigation runoff has been shown to contain plant pathogens (MacDonald et al, 1994b; Steadman et al, 1975). *Phytophthora* spp. are known to be important waterborne pathogens of these capture and recycle systems, and therefore *Phytophthora* is a logical target for disease management. Previous studies have shown that concentrations of *Phytophthora* in recycled irrigation water drawn from retention basins were considerably lower

than the concentrations found in the runoff entering the retention basins (Wilson, 1998). A solution to reusing captured runoff containing plant pathogens is to treat the captured water before reusing (Steadman et al, 1975). There are treatments available to disinfest irrigation water, including ultraviolet radiation, ozonation, chlorination, and heat treatment (MacDonald et al, 1994a). The purpose of this research is to understand the biological and physical processes occurring in retention basins that result in these declines. This information should lead to recommendations to the nursery industry for managing captured runoff to achieve the maximum reductions. Furthermore, managing captured runoff in retention basins to decrease pathogens provides less costly and more environmentally sound pathogen management options than those currently recommended.

Occurrence of *Phytophthora* pathogens in irrigated crops. Diseases caused by *Phytophthora* spp. are often associated with irrigated crops. *Phytophthora* spp. are a common cause of root and collar rot disease of apple trees and have been recorded in essentially all apple orchards world wide (Jeffers and Wilcox, 1990). In 1962 and 1963, McIntosh found *Phytophthora cactorum* in an irrigation source for a British Columbia apple orchard, in which major losses from *Phytophthora* collar rot had occurred. This study further showed that *P. cactorum* was a contaminant in the irrigation systems in the Okanagan and Similkameen Valleys in British Columbia. *P. citrophthora* and *P. parasitica* are known to be a major cause of root disease in irrigated citrus orchards in Arizona (Matheron et al, 1997). Zoospores released by these species can infect citrus root and bark tissue when favorable conditions of soil moisture and temperature occur (Matheron and Porchas, 1996). *Phytophthora spp.* were shown to have caused large losses of trees in irrigated commercial cherry orchards in California. These different *Phytophthora* spp. were isolated from the roots and bark of cherry trees with crown and root rot (Mircetich and Matheron, 1976). Runoff water entering retention ponds at three commercial California nurseries was tested for pythiacious fungi and *Phytophthora* propagules (MacDonald et al, 1994a). Many propagules of pythiacious fungi and several species of *Phytophthora*, which are known to be pathogenic to irrigated nursery crops were recovered. In surveys of irrigation ponds for

vegetables and ornamentals in southern Georgia in 1976 and 1977, many plant pathogens were recovered including *Pythium* spp., *Phytophthora* spp., as well as saprophytic fungi (Shokes and McCarter, 1979). Studies at Greenleaf Nursery have shown high levels of *Phytophthora* in irrigation runoff water (Wilson, 1998). Those studies showed that *Phytophthora* was recovered in many retention ponds and can be considered a major plant pathogen in irrigation systems.

Occurrence, survival, and motility of *Phytophthora* in irrigation water. Zoospores of *Phytophthora* spp. are the principal propagules in local dissemination and the major cause of new infections (Rana and Gupta, 1984). Preliminary studies in the Greenleaf Nursery system have shown that >94% of *Phytophthora* propagules recovered from runoff were zoospores, probably encysted zoospores (Wilson, 1998). Plant pathogens capable of surviving long enough to be recycled are of most concern. Irrigation water containing pathogens can contaminate soil (McIntosh, 1966). Various propagules of *P. aphanidermatum*, another zoosporic fungus, were found to maintain different survival rates in an irrigation pond (Shokes and McCarter, 1979). Thomson and Allen (1976) showed that zoospores remain motile up to 20 hours. They also discovered that zoospores survived in irrigation water between 40 and 60 days. Zoospore viability and motility of *Phytophthora* spp. have been studied in terms of ion concentration and ratio. According to these studies, Ca^{2+} concentrations suppressed the release of zoospores from either sporangia or zoospore cysts in *P. parasitica* (von Broembsen and Deacon, 1997). These researchers also found that calcium concentrations reduced zoospore motility and caused encystment. Application of Ca^{2+} to encysted *P. parasitica* zoospores inhibited the release of secondary zoospores (von Broembsen and Deacon, 1996).

Biology of *Phytophthora* zoospore cysts in irrigation water. Encysted zoospores have the potential for a more dynamic biological function. Zoospore cysts may not remain sedentary, and therefore their ability to reemerge becomes important. Diplanetism, repeated emergence, and indirect germination are all terms used to describe the release of a zoospore from an encysted zoospore. Diplanetism is defined as the condition in which there are two motile stages with a resting stage in between for zoospores in the Oomycetes (Ainsworth, 1961). Direct

germination refers to cyst germination by germ tube, whereas indirect germination refers to cyst germination by a zoospore (Blackwell, 1949). Salvin (1940) proposed that diplanetism was a means for further exploring the environment in search of a food source by continuing the motile phase. He observed a total of five successive swarmings of motile *Achyla* zoospores, which did not vary in size or shape except that the amount of material in the zoospore decreased with each swarming.

Observations of indirect germination of cysts to yield additional zoospores have been made in respect to temperature and the presence of nutrients. Direct germination of zoospore cysts by germ tubes was shown to be maximum at 25°C and by indirect germination at 15°C (Ho and Hickman, 1967). It was also observed that it took longer for release of secondary zoospores than direct germination. The duration for zoospore motility was longest at 15°C, but it diminished at extreme temperatures of 5 and 36°C. Von Broembsen and Deacon (1997) studied the effects of calcium at multiple stages on zoospore biology and found that the presence of $CaCl_2$ in aqueous solution significantly increased direct germination, but restrained the release of further zoospores by indirect germination.

Settling and dispersal of *Phytophthora* propagules in irrigation water. Understanding the settlement and dispersal of *Phytophthora* propagules in irrigation water will aid in developing management practices for capture and recycle systems. *Phytophthora* spp. have been isolated from irrigation water channels, and the dispersal of inoculum was found essentially in the direction of the flow of water (Neher and Duniway, 1992). The physical movement of spores of *Fusarium culmorum* was studied by Smither-Kopperl, Charudattan, and Berger (1998) by testing spore settlement in still water, spore dispersal from a point source, and spore dispersal in moving water. They determined that the main physical factors causing spore movement in still water are lateral dispersal and sinking due to the force of gravity, which accounts for the apparent dilution of the inoculum concentration. These same factors applied in moving water as well as in a chaotic environment.

When a small sphere descends due to the force of gravity through a viscous medium, it acquires a constant velocity according to Stokes' Law (Smither-Kopperl et al, 1998; Lynch and Hobbie, 1988). The terminal velocity of a smooth sphere with a diameter between 1-µm and 100-µm can be predicted by Stoke's Law (Gregory, 1973). The settling velocity of a single spherical particle of known density and diameter less than 60-µm can be theoretically deduced from the particle diameter by applying Stokes' Law (Lovell and Rose, 1988). The density of spores has been established to be between 1.1 and 1.2, however lower densities have been reported (Gregory, 1973; Lynch and Hobbie, 1988).

Retention time is the time required for a quantity of water to flow through a settling basin. For settling to occur, this time should be adequate so that all the particles of a selected size will settle to the bottom of the retention basin (Haman et al, 1989). Their studies for the design of a retention basin found that the inlet of a retention basin should reduce entrance velocities, limit turbulence, and distribute water uniformly across the basin.

CHAPTER II

BIOLOGY OF *PHYTOPHTHORA* SPP. IN IRRIGATION WATER

This chapter presents research on the basic biology of *Phytophthora* spp. in irrigation water. It focuses on propagule size distribution in irrigation runoff, on zoospore production and cyst germination in different lake waters, and on the phenomenon of repeated zoospore emergence or swarming in irrigation water. Understanding how zoospores function in different types of water under laboratory conditions should lead to understanding of their function in natural field conditions.

MATERIALS AND METHODS

Maintenance of experimental cultures. Cultures of *Phytophthora* spp. were maintained and stored on malt extract agar (MEA; 10 g Difco Malt Extract and 15 g Difco Bacto-Agar per liter distilled water) in McCartney bottles. Species used for experimental purposes were *P. cinnamomi*, *P. citricola*, *P. citrophthora*, *P. cryptogea*, and *P. parasitica* (Table 2.1), all of which had all been isolated from nurseries.

Production of zoospores. Cultures were grown on Cornmeal Agar (CMA; 17 g cornmeal agar per liter distilled water) for 2-3 days at 24°C. Cultures were then transferred to V8 agar (V8A; Ribeiro, 1978) and allowed to incubate at 24°C for 3-5 days. Ten 5-mm square blocks were transferred to 100 x 15 mm petri plates containing 10 ml clarified V8 broth and incubated at 24°C for 24 h. The V8 broth was removed with a pipette and discarded. The mycelial mats were rinsed twice with 10 ml mineral salt solution (MSS; 3.08 g $Ca(NO_3)_2 * 4 H_2O$; 1.49 g $MgSO_4 * 7 H_2O$; 0.51 g KNO_3 per liter distilled water), and incubated in 10 ml MSS per plate under fluorescent lights at room temperature. After 24 h the MSS was removed and replaced with 10 ml fresh MSS. After four days the MSS was removed and the mycelial mats

were washed twice with 10 ml sterile distilled water (SDW). SDW (11 ml) was added to each plate and plates were placed in a refrigerator at 5°C for 45 minutes. After 45 min the plates were removed from the refrigerator and allowed to incubate at room temperature (20°C ± 1°C) on the lab bench. Zoospores were released after 1-2 h. An aliquot of zoospore suspension was encysted by vortexing in an Eppendorf tube for 70 sec using a Maxi Mix II vortex machine. A SPotlite® hemacytometer (Baxter Healthcare Corporation, McGaw Park, IL) was used to estimate the concentration of zoospores

Assessment of mode of zoospore cyst germination. Suspensions of motile zoospores (usually 1 ml/ Eppendorf tube) were encysted by vortexing for 70 sec as previously described. Cyst germination was evaluated microscopically at various times after encystment. Cysts were resuspended by vortexing for 5 seconds and then 80-µl drops of suspension were placed on slides held in 150 x 20-mm Pyrex glass petri plates with moist paper towels to maintain wetness. One hundred cysts in each 80-µl were scored as ungerminated, germinated by germ tubes, or germinated by release of a single zoospore at 10X using a photomicrographic attachment (Nikon Inc. Instrument Group, Garden City, NY) and a video screen. Spores were considered to have germinated directly by germ tubes if the germ tube length exceeded the diameter of the cyst. Indirect germination by release of a motile zoospore was scored when cysts were empty (ghosts) and release pores were visible. Assessments were replicated for all treatments.

Determination of size classes of propagules in irrigation runoff. Samples of irrigation runoff were collected at Greenleaf Nursery Company from two sites, the Hub (sample RH) and the Snake Pit (sample RSP), to determine the size of *Phytophthora* propagules recovered throughout the season. Water samples (3.78 L) were taken over a 20 min time period from the flowing runoff water at these sites. Three class sizes for propagules were evaluated by filtering three replicate 50-ml sub-samples of irrigation water from each time period sequentially through a 177-µm sieve, a 20-µm sieve, and a 3.0-µm Nucleopore® polycarbonate membrane filter. This allowed for the propagules to be classed as greater than 177 µm, between 20 µm and 177 µm,

or less than 20 μm. Propagules remaining on the sieve at each filtration were washed from the sieves into 50-ml beakers with SDW from a wash bottle. Each sample was then filtered through a 3.0-μm Nucleopore® polycarbonate membrane filter. The filter was inverted and plated to a 60 x 15-mm petri plate containing selective media, P_{10}VPH (Ribeiro, 1978). After incubation for 24 h at 24° C, filters were removed and the plates were returned to incubation for another 24 h. Colony forming units were counted after the second 24 h of incubation.

Evaluation of zoospore production. The ability of certain *Phytophthora* isolates to produce large numbers of zoospores was evaluated to select working cultures representative of the species commonly found in irrigation runoff for use in experiments. Each culture was given a rating from * (no to few zoospores produced) to **** (abundant zoospore production) based on the visual production of zoospores and sporangia using a Stereoscope SMZ-2B/2T (Nikon Inc. Instrument Group, Garden City, NY) and a light microscope (Nikon Inc. Instrument Group, Garden City, NY).

Evaluation of zoospore production and cyst germination of *Phytophthora* in different water treatments. Zoospore production and cyst germination in different lake waters by *P. parasitica*, GLN 9-3, were evaluated to select lake waters to be used in further studies. Surface water was collected using a 3.78 L container at sites at Lake Carl Blackwell, Lake Sanborn, and Lake McMurtry, near Stillwater, OK, in the first series of experiments and at Lake Carl Blackwell and Lake Tenkiller, Cherokee County, OK for the second series of experiments. Temperature, pH, and electrical conductivity (EC) were recorded at time of sampling at each location using a Cole-Parmer® pH/mV/Temperature meter and Basic Conductivity Meter (Cole-Parmer Instrument Company, Vernon Hills, IL), and water was transported back to the laboratory. Lake water was filtered using a 3.0-μm Nucleopore® polycarbonate membrane filter held in a Nucleopore® filter funnel assemblage (Costar Scientific Corporation, Cambridge, MA) to remove any naturally occurring fungal propagules. Isolate GLN 9-3 was sporulated and zoospore release was induced in filtered lake water as previously described. SDW and reverse osmosis water (ROW) were the controls for the first and second

series of experiments, respectively. The first experiment evaluated zoospore production only and was duplicated. The second experiment evaluated both zoospore production and cyst germination, and was triplicated. One-ml aliquots were removed from each treatment plate, placed in separate Eppendorf tubes, and vortexed for 70 seconds to encyst. One tube was used for estimation of zoospore concentration using the hemacytometer method as described above, and in Experiment II the tube was similarly prepared and set aside to allow for germination to be assessed 3 h and 8 h later as described above.

Repeated emergence of zoospores from cysts. *Experiment I.* A suspension of motile zoospores was produced as described above. A total of 24 ml was harvested from several plates and combined. Sixteen 1-ml aliquots were each placed in separate Eppendorf tubes and vortexed for 70 seconds to encyst zoospores. The remaining 8 ml was placed in a McCartney bottle and encysted by vortexing for 2 minutes. The content of one Eppendorf tube was used to determine the concentration of cysts by hemacytometer counting. The McCartney bottle and other fifteen Eppendorf tubes were set aside to allow germination to take place. It has been previously shown that re-emergence of zoospores from cysts is completed by 7-8 h after encystment and that the majority of the negatively geotrophic motile zoospores congregate near the surface (von Broembsen and Deacon, 1997). Direct germination by germ tubes begins within the first hour and can be successfully scored after 2 h. Series in Eppendorf tube. At 8 h after encystment of the sporangial derived zoospores to form sporangial derived cysts (SDC), 0.25 ml was taken from the top of twelve of the tubes and four of these sub-samples were combined in each of three Eppendorf tubes. The contents of these tubes (cyst derived zoospores) were vortexed for 70 seconds to induce formation of cyst derived cysts (CDC1) and set aside for further assessment.

The sporangial derived cysts (SDC) in the remaining three tubes were then scored separately for percent germination for this time period (8 h after encystment) and discarded as described above. At 16 h, the remaining three Eppendorf tubes containing CDC1 were scored again for percent direct germination and percent indirect germination. The second generation

cyst derived zoospores were harvested by removing 0.33-ml aliquots from the top of each tube, placed into a single tube, and vortexed to cause encystment and this suspension of second generation cyst derived cysts (CDC2) was incubated overnight. Percent germination of CDC2 was calculated the following morning (18 h). All treatment assessments were carried out in triplicate.

Series in McCartney bottle. Two drops taken from the top of the suspension incubated in the McCartney bottle were examined microscopically at 8 h to confirm that motile zoospores were in the top portion at the end of the 8 h time period. The upper 3 ml of the suspension was put in a 60 x 15-mm petri dish. Ten freshly cut rhododendron leaf pieces, 4 mm x 12 mm, were floated in the dish. Three ml from the bottom of the vial were put in a separate petri dish and another 10 pieces of rhododendron leaves were floated. At two hours the leaf pieces were removed, and plated onto P_{10}VPH culture media. The plates were incubated at 24°C for 24 h. Ten more rhododendron leaf pieces were placed in the same dishes for 5 h, then assayed as described above.

Experiment II. Successive re-emergence was evaluated for four isolates of *Phytophthora* spp.: GLN 3-3 (*P. citricola*), 1D-A (*P. cinnamomi*), GLN 9-3 (*P. parasitica*), and GLN 7-23 (*P. citrophthora*). Zoospores were produced as described above for each isolate and the resulting sporangial derived zoospores were harvested. A 1-ml aliquot was used for hemacytometer counts as previously described. The remaining 20 ml were placed in a McCartney bottle, vortexed for 2 min to encyst the zoospores, and set aside to incubate. After 8 h, the upper 8 ml containing motile cyst-derived zoospores were removed, a 1-ml aliquot was scored for percent germination and the remainder of the suspension was vortexed to encyst the zoospores. After 13 h, generation two cyst-derived zoospores (CDZ2) were harvested, a 1-ml aliquot was scored for percent germination, and the remainder of the suspension was vortexed to encyst the motile zoospores. After 24 h, the remaining suspension was scored for germination. Treatment assessments were done in triplicate.

Infection of vinca seedlings. Vinca seedlings, Grape Cooler Series, were sown in Redi-Earth Peat-Lite Mix (Scotts-Sierra Horticultural Products Company, Marysville, OH) in 288-

plug plastic seedling trays. The trays were incubated in a growth chamber set for 12 h of light and 12 h of darkness at 28°C and 21°C, respectively. The plug trays were covered with moist paper towels. Once seedlings began to germinate, the moist paper towels were removed. Trays were irrigated daily with ROW as necessary. After three weeks of growth and 24 hours prior to inoculation, treatment blocks were prepared by cutting the plug trays to create blocks of 16 seedlings arranged four by four. Roots that had emerged from drain holes were cut from the bottom of each plug and seedling blocks were placed in the bottom of 100 x 100 x 15-mm square S/P® Brand diSPo® petri dishes. Each dish was labeled as to the treatment to be received.

The four treatments consisted of motile primary zoospores, encysted primary zoospores, motile secondary zoospores, and encysted secondary zoospores with concentrations of 10^3 cells/ml. There were four replicates per treatment. Primary treatments and secondary treatments were placed separately on trays in the growth chamber. The day of inoculation the plants were not irrigated so that the media would not be saturated and the plants could absorb the inoculum suspension. Inoculum was prepared from *P. parasitica* (GLN 9-3) zoospores released as described above. The suspensions were used as motile zoospores or encysted zoospores by vortexing and diluting to 10^3 cells/ml. The plants were flood inoculated by adding 20 ml of the specified treatment to each petri dish. The seedling blocks were then placed back in the dishes and held down firmly in the center for 10 sec to allow for even saturation. The secondary treatment received 20 ml SDW during inoculation of the primary treatments and the primary treatment received 20 ml SDW during the inoculation of the secondary treatments. Thereafter the seedling blocks were flood irrigated with SDW as needed over a 10 day period. Symptoms and deaths were recorded daily. After 10 days, the plant roots were washed in ROW and placed in labeled paper towels and plastic bags to prevent cross contamination. Under sterile conditions, each plant root was plated onto P_{10}VPH to detect infection by *P. parasitica*. The plates were observed after 24 h of incubation at 24° C. Positive and negative results were recorded. A second experiment had the same treatments with concentrations of 10^2 cells/ml and 10^3 cells/ml with three replicates per treatment.

RESULTS

Propagule size classes found in nursery irrigation runoff. Zoospores comprised greater than 94% of the *Phytophthora* propagules recovered in the class size of less than 20 μm over three sampling dates (Table 2.2). The August sampling was not included in this percentage because the assay plates had too many colony forming units per plate to count. Microscopic examinations of the plates showed that the numerous colony forming units in the RSP sample of propagules between 20 μm and 177 μm on August 4, 1999, were due to sporangia releasing zoospores on the assay plates. Given the high percentage of propagules in the less than 20 μm size class, zoospores were selected as the propagule on which to focus in subsequent studies.

Evaluation of zoospore production. One isolate of each of five *Phytophthora* spp. commonly isolated from nursery runoff was selected for further study based on its ability to produce zoospores in laboratory culture. A complete list of the twenty-one isolates evaluated is shown in Table 2.1. One isolate of each species with the highest rating was chosen: GLN 3-3, *P. citricola* from nursery irrigation runoff; 1D-A, *P. cinnamomi* from rhododendron; GLN 9-17, *P. parasitica* from nursery irrigation runoff; GLN 7-23, *P. citrophthora* from nursery irrigation runoff; II-15, *P. cryptogea* from nursery off-site surface water; and GLN 9-3, *P. parasitica* from nursery irrigation runoff. These isolates were chosen based on comparisons made within a group of isolates for individual species (Table 2.3). Since the amount of zoospore production is different for *Phytophthora* spp., the isolates of each species chosen for use in experiments did not typically produce equivalent numbers of zoospores in experiments.

Evaluation of zoospore production and cyst germination of *Phytophthora parasitica* in different water treatments. In Experiment I, *P. parasitica*, isolate GLN 9-3, produced zoospores equally well in all three lake waters (Lake Carl Blackwell, Lake McMurtry, and Lake Sanborn) as compared with SDW (Table 2.4). In Experiment II, zoospore production in two lake waters (Lake Carl Blackwell and Lake Tenkiller) was equal to or better than zoospore production in ROW (Table 2.5). In this experiment germination by germ tubes or by release of zoospores in LCB or GLN was not significantly different from ROW controls. Overall, results show

that any of the waters were suitable for further experiments requiring large numbers of zoospores. Therefore, Lake Carl Blackwell was chosen for further studies requiring lake water because of its to the convenient location only 10 miles away.

Repeated emergence of zoospores. *Experiment I.* When looking at generations of repeated emergence in *P. parasitica* (GLN 9-3), three generations of cyst derived zoospores were scored for percent germination and a fourth generation was observed also (Fig. 2.1). *P. parasitica* was released in SDW and germination by re-emergence of the first, second, and third generations of cyst derived zoospores was 23.7, 66.3, and 74.7%, respectively.

Experiment II. In a further experiment, germination of four *Phytophthora* spp. by zoospores ranged from 24.5 to 72.8% for sporangial derived zoospore cysts (Fig. 2.2) and from 43.3 to 62.0% for first generation cyst derived zoospore cysts (Fig 2.3).

Infection of vinca seedlings. In the first experiment, flood inoculation with 10^3 zoospores/ml caused infection of 255/256 or 99.6% of the plants (data not shown). Therefore, no significant difference was seen between seedlings inoculated with motile sporangial derived zoospores, motile cyst derived zoospores, encysted sporangial derived zoospores, and encysted cyst derived zoospores. In the second experiment, flood inoculation with 10^2 and 10^3 zoospores/ml caused infection of 162/192 or 84.4% of the plants and 184/192 or 95.8% of the plants, respectively (Fig. 2.4). No significant difference was found among the four treatments. There was no significant difference between the motile sporangial derived zoospores and the motile cyst derived zoospores at 10^2 or 10^3 zoospores/ml. Likewise, there was no significant difference between the encysted sporangial derived zoospores and the encysted cyst derived zoospores at 10^2 or 10^3 zoospores/ml.

DISCUSSION

This research presents new information about the biology of the predominant *Phytophthora* propagule in irrigation water, the zoospore in its motile and encysted state. The establishment of the zoospore as the principal propagule in irrigation water led to further studies

in search of understanding the role of these zoospores in the survival of *Phytophthora*, particularly in retention basins.

Establishing the methods for production of zoospores and modes of germination in isolates commonly found in irrigation water led to the selection of several isolates for studies in lake waters. Not all species produce equivalent numbers of zoospores, and also there is great variation among isolates of a species. Studying several species helps to get an overall picture of *Phytophthora* spp. Release of zoospores and modes of germination in different lake waters is valuable information because it relates to what is taking place in retention basins. These studies assessed modes of germination for three generations, which is the first demonstration of multiple generations in *Phytophthora* spp.

The infection of vinca seedlings by sporangial derived and cyst derived zoospores was tested to evaluate the ability of re-emerged zoospores to infect. It was found that cyst derived zoospores are as pathologically fit and competent as sporangial derived zoospores. This demonstrates the significance of the cyst derived zoospore to the nursery industry and its implications when looking at management strategies for the control of *Phytophthora* in retention basins. Quantification of modes of germination by several *Phytophthora* spp. in different types of water was recorded, and these findings regarding modes of germination offer a totally new perspective on what is actually occurring in irrigation water. This research focuses on the implications for stored water when recycling irrigation water.

Table 2.1 Isolates screened for zoospore production to select cultures to be used for subsequent studies.

Species	Isolate	Host / Isolated from
P. citrophthora	G98 2-3	GLN Irrigation Runoff
	5A-2G	Rhododendron
	GLN 7-23	GLN Irrigation Runoff
P. citricola	5G-R-1	Dogwood
	GLN 3-3	GLN Irrigation Runoff
	GLN 6-1	GLN Irrigation Runoff
	P065	Hibiscus
P. cinnamomi	1D-A	Rhododendron
	5N-L	Dogwood
	P022	*Leucadendron comosum*
P. cryptogea	GLN 10-5	GLN Irrigation Runoff
	II-15	GLN Off-Site Surface Water
	GLN 8-19	GLN Irrigation Runoff
	GLN 9-1	GLN Irrigation Runoff
	GLN 9-16	GLN Irrigation Runoff
	G99 3-5	GLN Irrigation Runoff
P. parasitica	GLN 9-17	GLN Irrigation Runoff
	P068	*Catharanthus roseus*
	GLN 9-3	GLN Irrigation Runoff
	GLN 9-5	GLN Irrigation Runoff
	II-16	GLN Off-Site Surface Water

Table 2.2 Mean colony forming units of three propagule size classes over four sampling dates in 1999 at sampling sites RH and RSP.

Site	Size Class	Mean colony forming units per liter[a]			
		Sampling Dates			
		6/3	7/8	8/4	9/22
RH	< 20 μm	154	274	TNTC[b]	160
RH	20 μm < x < 177 μm	0	20	0	6
RH	> 177 μm	0	6	0	0
RSP	< 20 μm	180	1286	TNTC	6
RSP	20 μm < x < 177 μm	34	66	566[c]	0
RSP	> 177 μm	0	14	20	0

[a] = based on three replicate samples
[b] = too numerous to count
[c] = sporangia released zoospores on the assay plate

Table 2.3 Evaluation of sporulation and zoospore production for selecting isolates used for experimental purposes.

Species	Isolate	Rating	Comments
P. citricola	5G-R-1	**	Good sporangia and zoospores
	GLN 3-3	****	Abundant sporangia and zoospores
	GLN 6-1	**	Average sporangia and zoospores
	PO65	*	Few sporangia; no zoospores
P. cinnamomi	1D-A	***	Good sporangia and zoospores
	5N-L	*	Poor sporangia; no zoospores
	PO22	*	Good sporangia; few zoospores
P. parasitica	GLN 9-17	****	Abundant sporangia and zoospores
	PO68	****	Abundant sporangia and zoospores
	GLN 9-3	****	Abundant sporangia and zoospores
	GLN 9-5	*	Poor sporangia with little openings
	II-16	****	Average sporangia and zoospores
P. cryptogea	GLN 10-5	*	Abundant sporangia; few zoospores
	II-15	***	Average sporangia; good zoospores
	GLN 8-19	*	Average sporangia; few zoospores
	GLN 9-1	*	Few sporangia or zoospores
	GLN 9-16	*	Very few sporangia or zoospores
	G99 3-5	-	Not *P. cryptogea*
P. citrophthora	G98 2-3	****	Abundant sporangia and zoospores
	5A-2G	****	Abundant sporangia and zoospores
	GLN 7-23	****	Abundant sporangia and zoospores

Table 2.4 Mean zoospore release in lake waters by *Phytophthora parasitica* isolate GLN 9-3.

Treatment	Zoospore Production[a]
Lake Carl Blackwell	1.50×10^5
Lake McMurtry	1.75×10^5
Lake Sanborn	1.17×10^5
Sterile Distilled Water	7.05×10^4
Mean	1.28×10^5
5% LSD	7.56×10^4

[a] = means of duplicate samples

Table 2.5 Mean zoospore release and cyst germination in lake waters by *Phytophthora parasitica* isolate GLN 9-3.

		Germination at 8 h[a]	
Treatment	Zoospore Production[a]	Germ tube	Zoospore
Lake Carl Blackwell	2.32×10^4	2.3	57.3
Greenleaf Nursery	1.09×10^4	5	53
Reverse Osmosis Water	7.59×10^3	7.3	57.7
Mean	1.39×10^4	4.9	56
5% LSD	4.14×10^3	9.14	19.45

[a] = means of triplicate samples

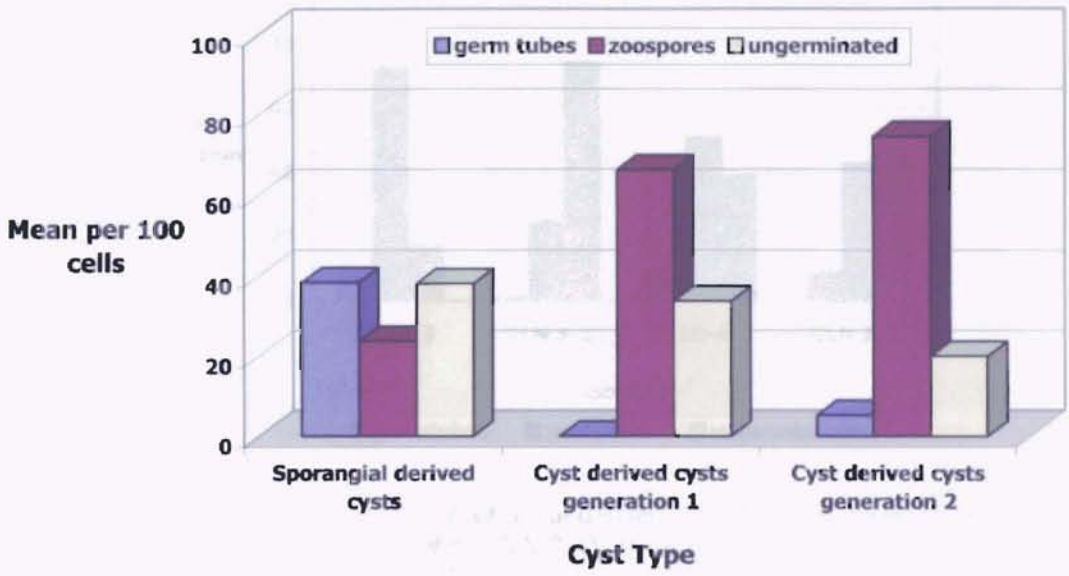

Fig. 2.1 Germination of three generations of zoospore cysts of *Phytophthora parasitica*, GLN 9-3.

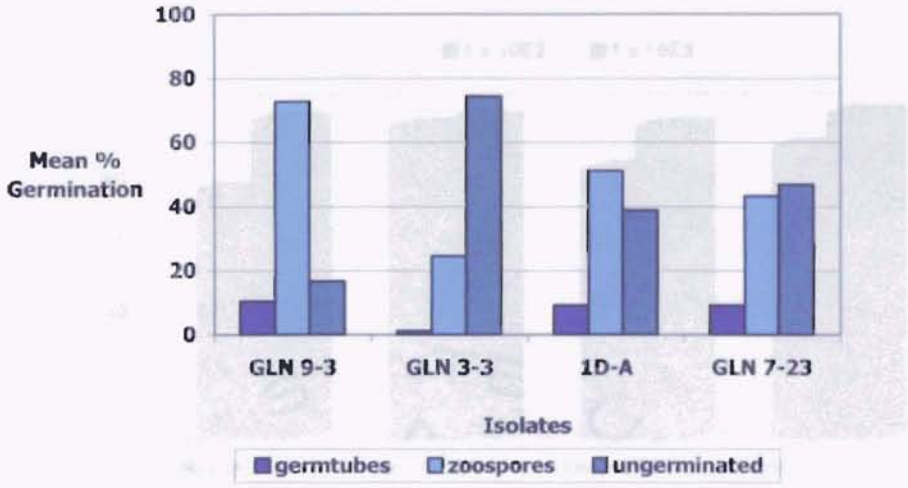

Fig. 2.2 Percent germination of sporangial derived cysts by germ tubes or by release of zoospores of *P. parasitica* (GLN 9-3), *P. citricola* (GLN 3-3), *P. cinnamomi* (1D-A), and *P. citrophthora* (GLN 7-23).

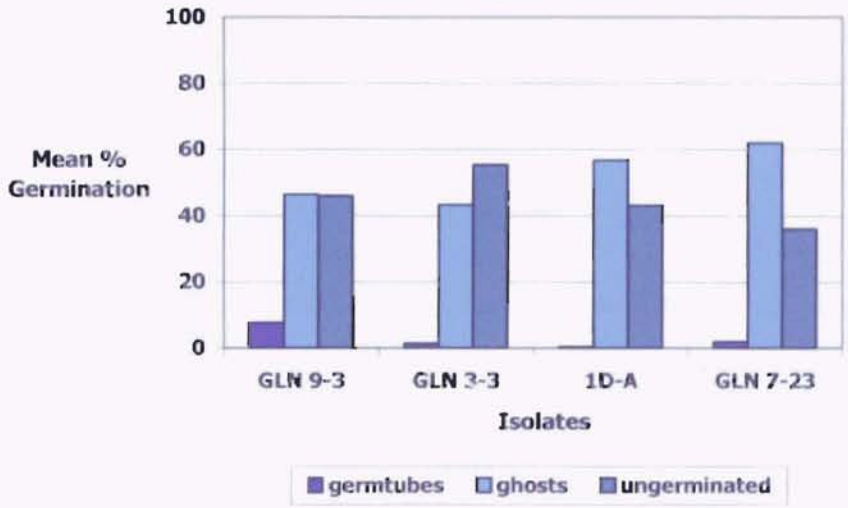

Fig. 2.3 Percent germination of cyst derived cysts by germ tubes or by release of zoospores of *P. parasitica* (GLN 9-3), *P. citricola* (GLN 3-3), *P. cinnamomi* (1D-A), and *P. citrophthora* (GLN 7-23).

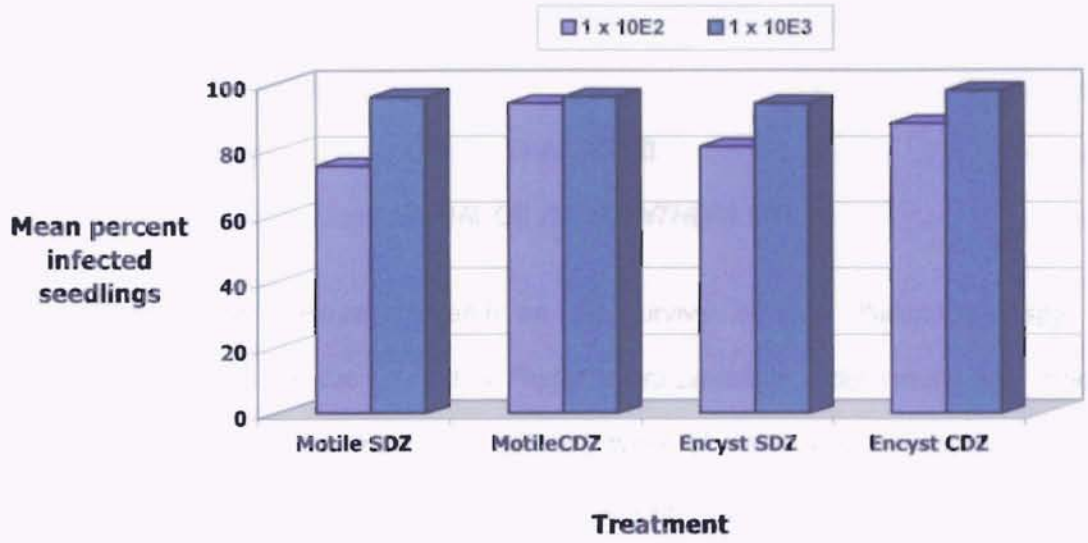

Fig. 2.4 Mean percent infection of vinca seedlings flood inoculated at 10^2 and 10^3 zoospores/ml of motile or encysted sporangial and cyst derived zoospores of *P. parasitica* isolate GLN 9-3. Three replicates of 16 seedlings were used per treatment.

CHAPTER III

SURVIVAL OF *PHYTOPHTHORA* SPP.

This chapter presents research on the survival of four *Phytophthora* spp. under laboratory conditions and the survival of *Phytophthora parasitica* under natural field conditions. The viability of encysted zoospores in different types of water was investigated to evaluate potential effects of water source and quality on survival. Field studies were also carried out to determine the influence of the natural environment on the viability of encysted zoospores at several sites and several times during a growing season. This information on survival of zoospores of *Phytophthora* spp. could be applied to managing the water within retention basins in nursery capture and recycle irrigation systems.

MATERIALS AND METHODS

Survival of *Phytophthora* spp. under laboratory conditions. Encysted zoospores of several *Phytophthora* spp. were stored in three treatment waters to assess the survival rate under laboratory conditions. The treatments were sterile distilled water (SDW), filtered lake water (FLW) from Lake Carl Blackwell, and double filtered lake water (DFLW) from Lake Carl Blackwell. Filtered water was filtered through a 3.0-μm Nucleopore® polycarbonate membrane filter as previously described. Double filtered lake water was filtered in the same manner as the filtered lake water and then filtered again through a 0.22-μm filter (Micron Separations Inc., Westborough, MA). Each treatment had three replicates.

Zoospores of four *Phytophthora* spp. (GLN 3-3, *P. citricola*, 1D-A, *P. cinnamomi*, GLN 9-3, *P. parasitica*, and GLN 7-23, *P. citrophthora*) were produced as described previously. The concentration of zoospores was estimated using a hemacytometer and the suspensions were diluted to approximately 10^3 zoospores/ml by addition of sterile distilled water. An additional

dilution to 10^2 zoospores/ml was accomplished by adding three ml of the original dilution to 27 ml of the treatment solution for a total of 30 ml in labeled glass medicine bottles and placed in a dark location at room temperature (20°C ± 1°C) (Fig. 3.1). The treatments were sampled immediately (zero time) and at several other time intervals afterwards. Each treatment was replicated three times for each isolate. To assess survival of propagules, medicine bottles were agitated to re-suspend propagules and 1 ml was removed and placed onto 60 x 15-mm petri plates containing P_{10}VPH selective media. The plate was swirled gently to distribute the sample evenly over the surface. Colony forming units were counted after 24 h of incubation at 24° C.

Survival of encysted zoospores of *Phytophthora* spp. under laboratory conditions consisted of four separate experiments. *Experiment I.* Four species, GLN 3-3, GLN 7-23, and 1D-A, and two water treatments, SDW and FLW, were used. Sampling times were 0, 7, 14, 21, and 35 days.

Experiment II. The second experiment consisted of three isolates, GLN 3-3, GLN 7-23, and 1D-A. Three treatment waters, SDW, FLW, and DFLW, were used. The sampling times were 0, 7, 14, 21, and 28 days.

Experiment III. The third experiment had the same species as Experiment II, with the addition of isolate GLN 9-3, *P. parasitica*, and only two water treatments, SDW and FLW. The sampling times were 0 h, 48 h, 1 week, and 2 weeks.

Experiment IV. The fourth experiment was the same as the third experiment, but an additional sampling time of 24 h was added.

Survival of *P. parasitica* in field conditions. Encysted zoospores of *P. parasitica* were enclosed in nylon mesh spore cages and placed in natural conditions to assess the survival rate. Field locations were Lake Carl Blackwell (Fig 3.2) and Greenleaf Nursery Company. The storage basin and the hub basin were chosen for the locations of the field survival studies at the Greenleaf Nursery site (Fig. 3.3).

Zoospores of *Phytophthora parasitica* (GLN 9-3) were released as described previously. The concentration of zoospores was estimated using a hemacytometer and dilutions were made

as necessary. Treatments consisted of several exposure times. In order to have countable numbers of propagules throughout population declines, three concentrations (10^3, 10^4, and 10^5 zoospore cysts/ml) were used. One ml of encysted zoospores was placed in each triplicate spore cage enclosing the zoospore cysts while exposing them to ambient water conditions. Each spore cage was made of a 5.08-cm PVC coupler and a 5.08 x 3.81-mm PVC reducer with two 2.97 cm diameter circles of 1-μm Nitex mesh (Sefar America Inc., Kansas City, MO) held between the coupler and reducer (Fig. 3.4). The encysted zoospores were pipetted onto one mesh circle, the second mesh circle was placed on top of the first. Then the coupler was securely pushed onto the reducer to hold both circles in place. The spore cages were then placed in color-coded 61 x 91-cm mesh utility bags representing the different concentrations. The mesh bags were placed in a cooler for transport. At the test sites, the mesh bags were tied together and placed into the designated lake or basin with a floating plastic container to suspend approximately 1 m below the surface of the water (Fig. 3.5).

Three cages were taken from each mesh bag at random for each concentration at each sampling time. The Lake Carl Blackwell samples were placed in gallon Ziploc® bags and transported back to the lab for assay. For the Greenleaf Nursery samples, P_{10}VPH plates were transported to the site so that the mesh circles could be plated out in a laboratory on location due to the long distance from the O.S.U. laboratory.

Survival was determined by plating each circle of mesh per spore cage onto separate 100 x 15-mm plates of P_{10}VPH. The mesh was removed after 24 h of incubation at 24° C and the plates were returned to the incubator for another 24 h. Colony forming units were counted after the second 24 h of incubation. Control samples were plated onto P_{10}VPH in the lab at 0 h for each concentration. Sampling occurred at 24 h, 48 h, and 1 week. Before sampling, pH and temperature of basin or lake water at each site were measured as described in Chapter II (Fig. 3.6). The time period of the field survival experiments extended throughout the growing season. The studies were conducted in the spring (beginning April 13, 2000), the summer (beginning June 6, 2000; beginning July 11, 2000), and the fall (beginning October 19, 2000).

Survival of *Phytophthora* spp. under laboratory conditions. *Experiment I.* The survival of encysted zoospores of three *Phytophthora* spp. in SDW and FLW over a 35 d period is shown in Table 3.1. The data as given in Table 3.1 are shown for comparison of species. Statistical analysis was not possible since initial densities were too high to count. Therefore in subsequent experiments the concentrations of the treatments were adjusted to be 10^2 zoospores/ml so that plates could be counted.

Experiment II. In this experiment of three isolates in FLW, sampling times extended over four weeks (Fig. 3.7). Mean colony forming units dropped drastically in the first week, and by the fourth week there were no colony forming units for any species or treatment. A drastic drop in colony forming units occurred within the first week for all three species in FLW. The DFLW treatment was used to determine if the absence of bacteria was a factor in the survival rate. The second filtration did not affect significant difference, and therefore DFLW was not used in further studies.

Experiment III. The mean percent survival rate of four species in SDW (Fig. 3.8), declined rapidly within the first 48 h. Survival rate in SDW declined to 35.0% for GLN 3-3, 11.0% for GLN 7-23, 3.7% for 1D-A, and 0.3% for GLN 9-3 within 48 h.

Experiment IV. The mean percent survival rate of four species in SDW and FLW (Table 3.2), showed repeated results as in previous experiments. There was a rapid decline in viability within 48 h. The percent survival rate in SDW declined to 20.6% for GLN 3-3, 13.5% for GLN 7-23, 5.5% for 1D-A, and 24.9% for GLN 9-3 within 48 h. The percent survival rate in FLW declined similarly to 10.0% for GLN 3-3, 10.4% for GLN 7-23, 6.0% for 1D-A, and 3.6% for GLN 9-3 within 48 h.

Survival of *P. parasitica* in field conditions. There was a consistent rapid decline within 48 h for all sites and dates of exposure (Table 3.3). Loss of viability of *P. parasitica* zoospore cysts in Lake Carl Blackwell beginning on April 13, 2000 is shown in Fig. 3.9, with an extinction rate of 0.0198/day and on June 6, 2000 (Fig. 3.10) with an extinction rate of

1.8476/day and 1.7987/day. The viability of *P. parasitica* zoospore cysts declined similarly at Greenleaf Nursery Company in the storage basin and in the hub basin (Fig. 3.11 and Fig. 3.12, respectively) beginning on July 11, 2000. The decline was a function of time during the first 48 h (slopes given in Fig. 3.9, 3.10, 3.11, and 3.12).

DISCUSSION

Several types of waters were used to evaluate the effect of water source and the potential influence of differences in microbial water quality on the viability of encysted zoospores of several *Phytophthora* spp. in stored water. These included sterile distilled water, lake water filtered to 0.22 μm to remove bacteria, and several different sources of lake water filtered to 0.3 μm to remove only larger microorganisms, such as naturally occurring *Phytophthora* propagules. Decline in viability was not affected by water source or water quality with respect to the presence of microorganisms.

As shown in Chapter II, zoospore production by *Phytophthora* spp. under laboratory conditions varies considerably both within and amongst species. This means that even though the best zoospore producing isolates were selected for use in the laboratory survival studies, there were still large differences in the amounts of zoospores available for these comparative studies. Despite these differences in initial units, the rates of decline during the first 48 h were very similar in all types of water.

The field studies were carried out over the growing season from the spring, through summer, and into the fall. Rates of decline during an exposure period were fairly consistent throughout the season, establishing that rapid loss of viability occurred in the first 24 to 48 h as predicted by laboratory studies. There seemed to be no apparent influence on survival by factors such as temperature and pH, which tend to fluctuate throughout the seasons.

These results demonstrate a relatively rapid natural decline in viability of encysted zoospores in stored water. Viability declined markedly within 24 to 48 h under both laboratory and field conditions. This suggests that increasing retention times will allow natural reductions of

pathogen concentrations in captured runoff. Management should thus be focused on ways to increase retention time in basins so that pathogens are reduced in recycled irrigation water.

Table 3.1 Mean[a] colony forming units in lab survival studies in treatments of sterile distilled water and filtered lake water.

Sterile Distilled Water:

Isolate	Time held (days)				
	0	7	14	21	35
GLN 3-3	TNTC[b]	TNTC	TNTC	10.3	0
GLN 7-23	TNTC	TNTC	TNTC	3.3	0
1D-A	102.7	12	27.67	41	5

Filtered Lake Water:

Isolate	Time held (days)				
	0	7	14	21	35
GLN 3-3	TNTC	TNTC	TNTC	1.3	0
GLN 7-23	TNTC	TNTC	TNTC	4.7	0
1D-A	57	TNTC	21	2.7	0

[a] = mean of three replicate samples
[b] = too numerous to count

Table 3.2 Summary of laboratory survival of encysted zoospores of four *Phytophthora* spp. in treatments of sterile distilled water and filtered lake water.

Sterile Distilled Water:

Isolate		Time held			
		0 h	24 h	48 h	1 wk
GLN 3-3		71	46	23	0
		78	32	12	0
		89	41	14	16
	mean	79.3	39.7	16.3	5.3
GLN 7-23		137	21	16	3
		113	27	18	0
		100	26	13	0
	mean	116.7	24.7	15.7	1
1D-A		80	22	6	0
		83	15	5	1
		71	17	2	1
	mean	78	18	4.3	0.7
GLN 9-3		48	18	11	0
		51	29	9	0
		42	32	15	0
	mean	47	26.3	11.7	0

5% LSD = 11.4
$\rho < 0.001$

Filtered Lake Water:

Isolate		Time held			
		0 h	24 h	48 h	1 wk
GLN 3-3		87	20	8	0
		83	34	6	0
		81	18	11	0
	mean	83.7	24	8.3	0
GLN 7-23		122	21	17	0
		113	27	9	2
		111	26	10	3
	mean	115.3	24.7	12	1.7
1D-A		88	3	7	0
		76	13	2	0
		87	6	6	0
	mean	83.7	7.3	5	0
GLN 9-3		60	14	4	0
		59	9	2	0
		48	5	0	0
	mean	55.7	9.3	2	0

5% LSD = 7.7
$\rho < 0.001$

Table 3.3 Summary of field survival of encysted zoospores of *P. parasitica* (GLN 9-3) exposed in mesh cages at Lake Carl Blackwell (LCB) and two Greenleaf Nursery (GLN) sites during the 2000 growing season. Recovery at each sampling time is given as the mean cfu/ml from three replicate cages.

Date	Location	Original concentration[a]		Time			
				0 h Mean cfu/ml[b]	24 h Mean cfu/ml	48 h Mean cfu/ml	1 wk Mean cfu/ml
04/13	LCB	10^3		122	17	-	3
				-[c]	7	-	1
				-	18	-	3
			mean	122 100% (90)	14 11.5% (19.4)	-	2.3 1.9% (8.1)
06/06	LCB	10^3		77	2	0	0
				47	5	1	0
				93	15	3	0
			mean	72.3 100% (90)	3 4.1% (11.5)	1.3 1.8% (8.1)	0 0% (0)
	LCB	10^4		163	73	1	0
				158	37	6	0
				-	35	3	0
			mean	160.5 100% (90)	48.3 30.1% (33.2)	3.3 2.1% (8.1)	0 0% (0)
07/11	GLN storage	10^3		237	112	38	-
				289	59	27	-
				253	53	13	-
			mean	259.7 100% (90)	74.7 28.8% (32.6)	26 10% (18.4)	-
	GLN hub	10^3		237	85	35	-
				289	41	12	-
				253	44	16	-
			mean	259.7 100% (90)	56.7 21.8% (28)	21 8.1% (16.4)	-
10/19	LCB	10^3		266	1	0	0
				215	1	0	0
				239	0	0	0
			mean	240 100% (90)	0.7 .29% (0)	0 0% (0)	0 0% (0)

[a] = cells/ml based on hemacytometer counts
[b] = mean cfu/ml are followed by mean percent recovery and arc sin tranformation values are shown in ( )
[c] = not sampled

Fig. 3.1 Medicine bottles used in laboratory experiments evaluating survival of encysted zoospores.

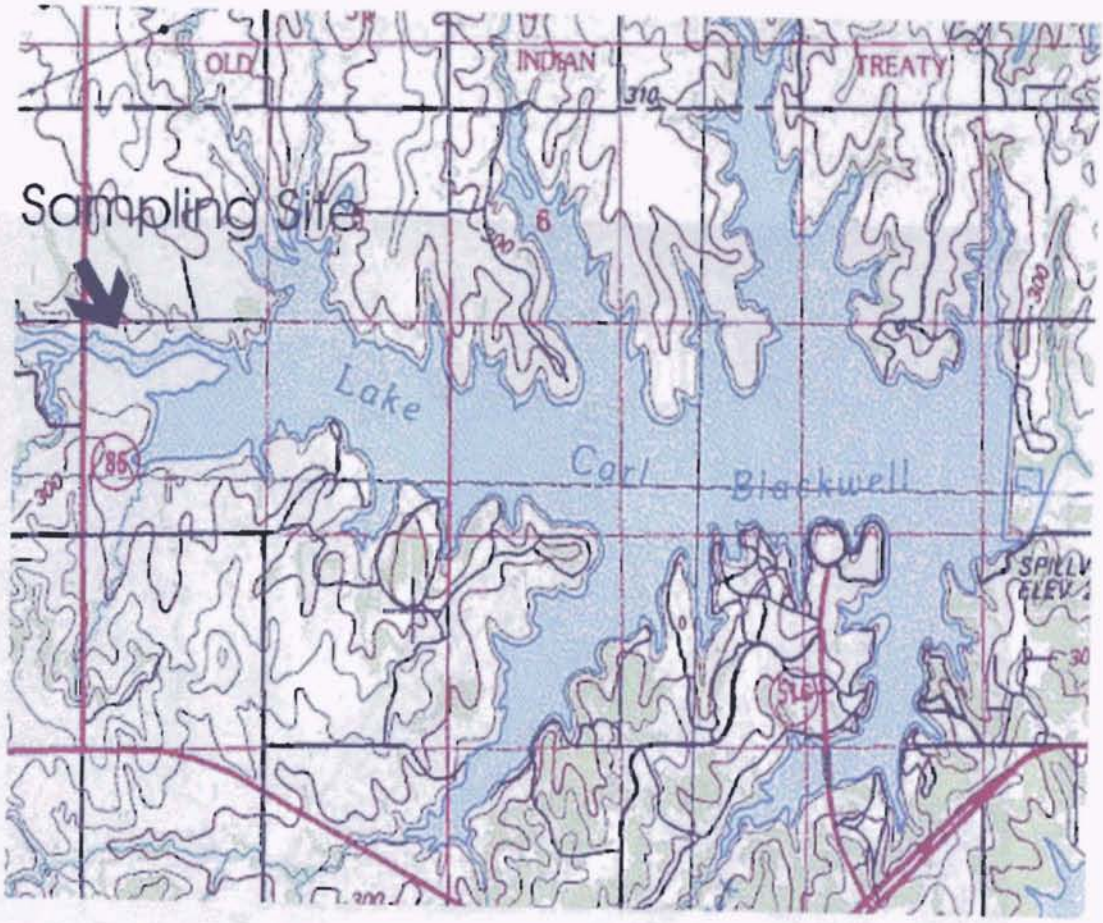

Fig. 3.2 Map of Lake Carl Blackwell showing sampling location for field survival studies.

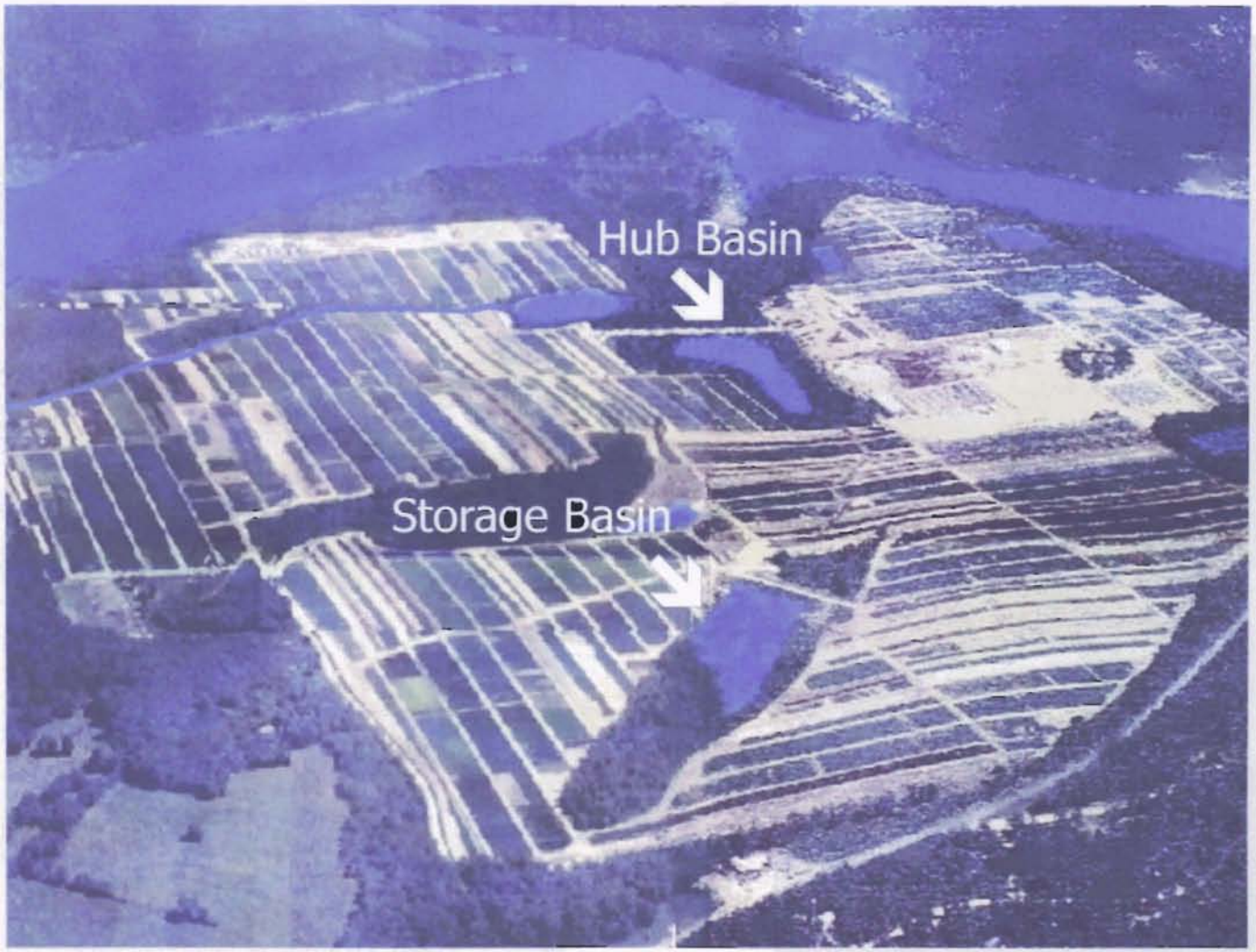

Fig. 3.3 Aerial view of GreenLeaf Nursery showing storage and hub basins for field survival studies.

Fig. 3.4 Spore cages made of PVC couplers and reducers with Nitex mesh.

Fig 3.5 Field survival sampling method using mesh utility bags and spore cages.

Fig. 3.6 Field sampling method at Greenleaf Nursery in the storage basin.

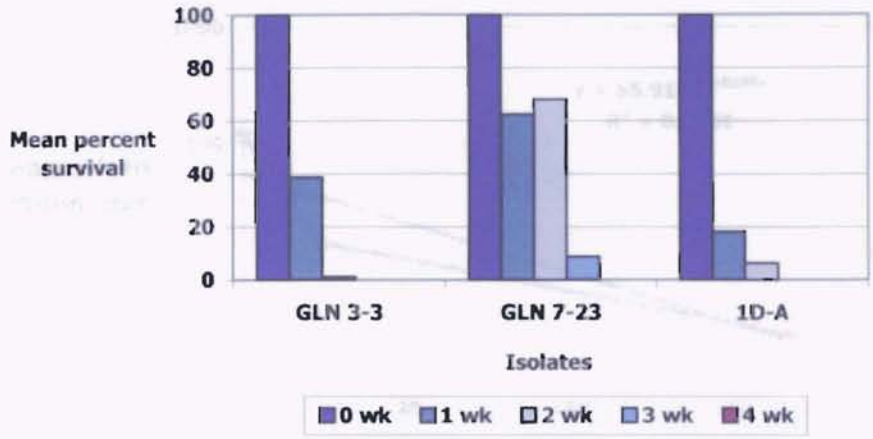

Fig. 3.7 Mean percent survival of encysted zoospores of *P. citricola* (GLN 3-3), *P. citrophthora* (GLN 7-23), and *P. cinnamomi* (1D-A) in filtered water from Lake Carl Blackwell under laboratory conditions over a four-week sampling period.

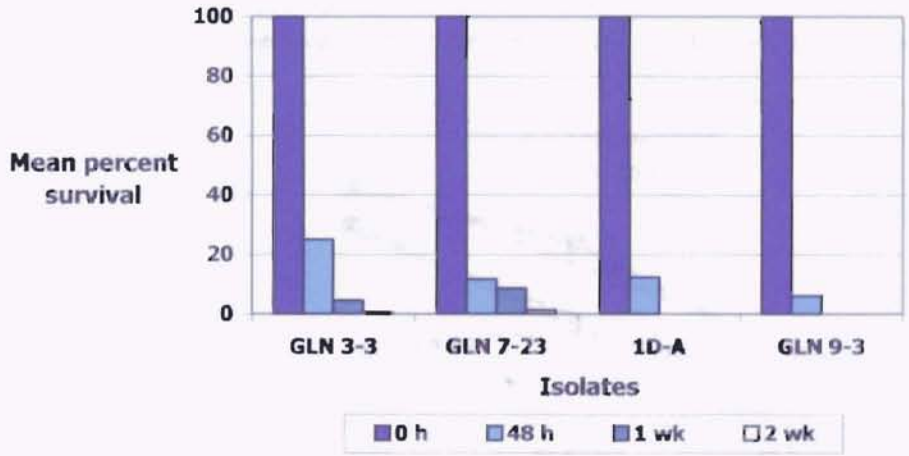

Fig. 3.8 Mean percent survival of encysted zoospores of *P. citricola* (GLN 3-3), *P. citrophthora* (GLN 7-23), *P. cinnamomi* (1D-A), and *P. parasitica* (GLN 9-3) in sterile distilled water under laboratory conditions over a two-week sampling period.

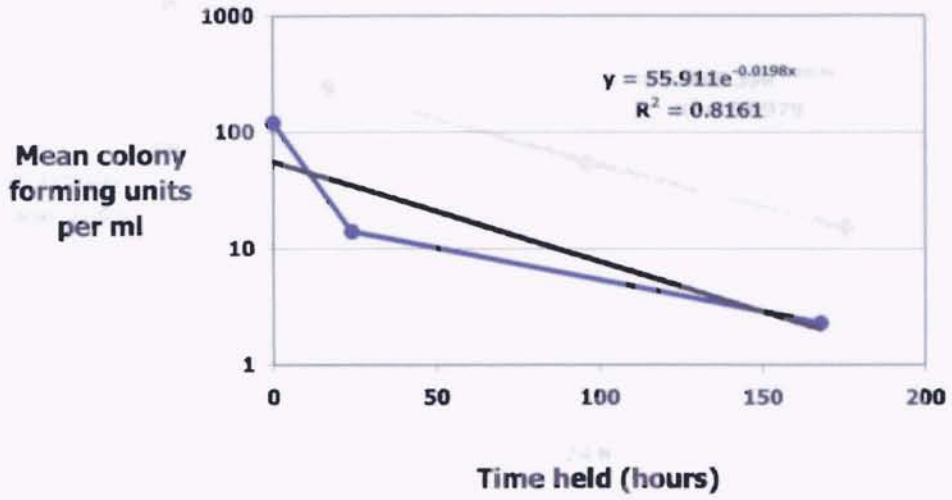

Fig. 3.9 Mean survival rate of encysted zoospores of *P. parasitica* in Lake Carl Blackwell beginning on April 13, 2000.

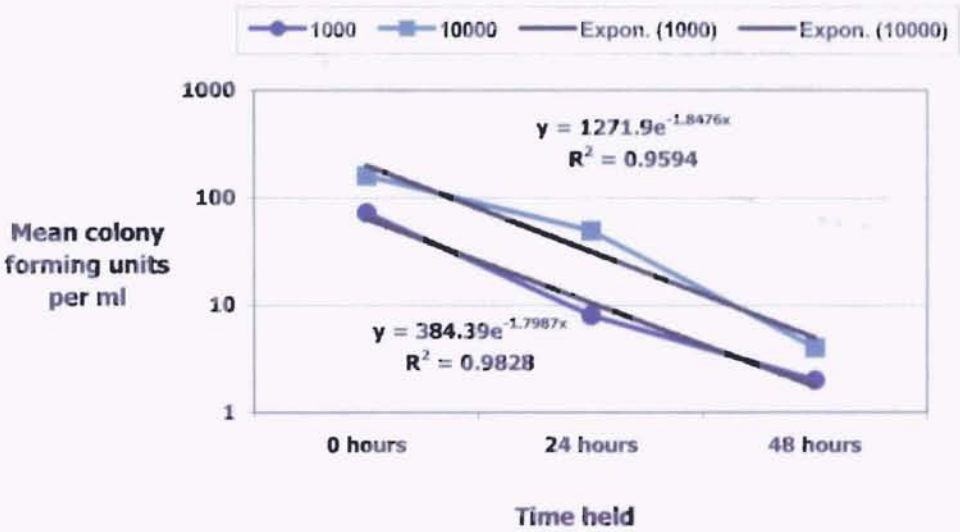

Fig. 3.10 Mean survival rate of encysted zoospores of *P. parasitica* from initial concentrations of 10^4 and 10^5 cells/ml in Lake Carl Blackwell beginning on June 6, 2000.

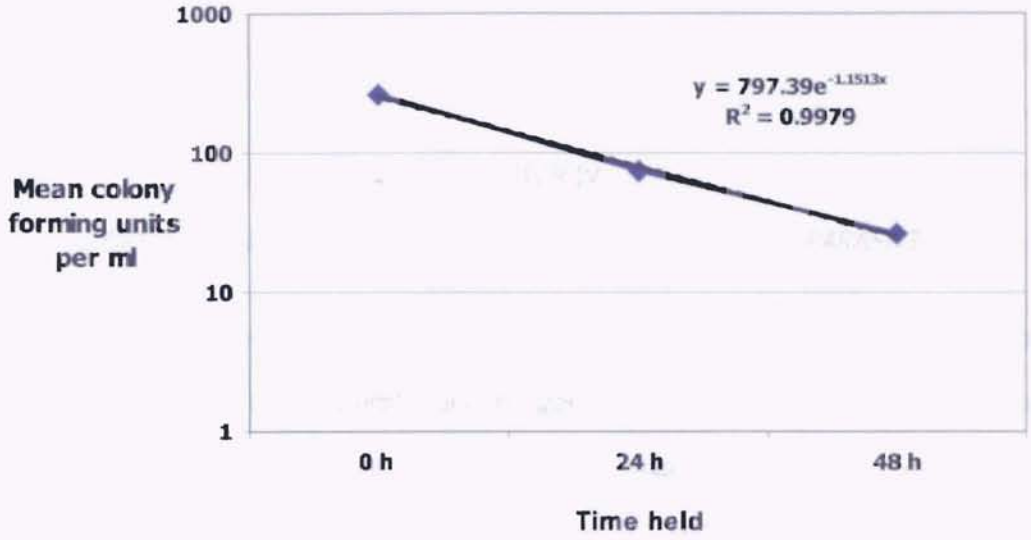

Fig. 3.11 Survival rate of encysted zoospores of *P. parasitica* at Greenleaf Nursery Company in the storage basin beginning on July 11, 2000.

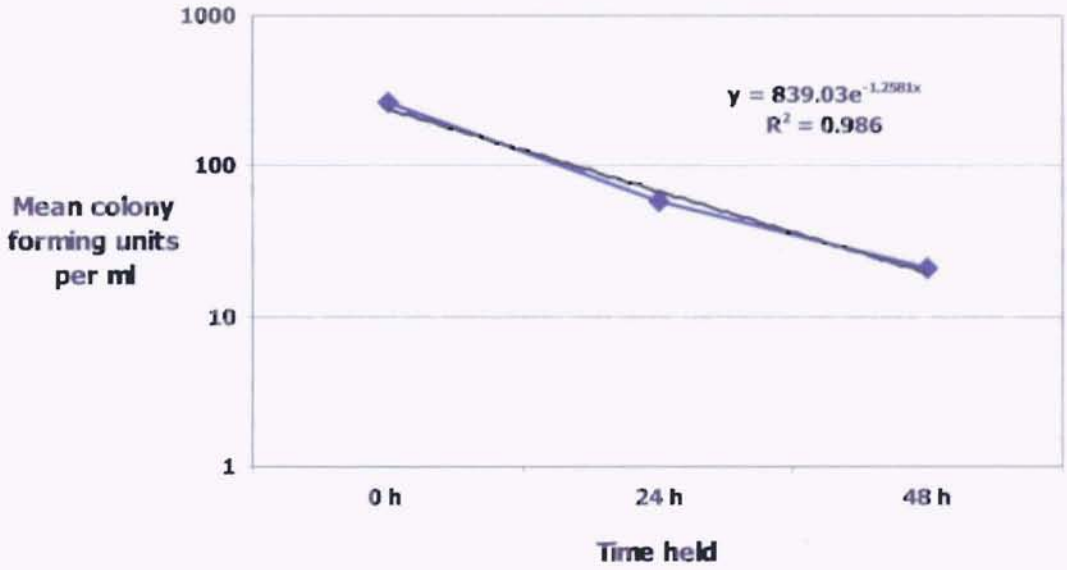

Fig. 3.12 Survival rate of encysted zoospores of *P. parasitica* at Greenleaf Nursery Company in the hub basin beginning on July 11, 2000.

CHAPTER IV

SETTLING AND LATERAL DISPERSAL OF *PHYTOPHTHORA PARASITICA*

This chapter presents research on the physical dispersal of encysted zoospores of *Phytophthora parasitica* both vertically and horizontally in still water. Studies evaluated the settling effect of cysts in water that may be similar to what is occurring in retention basins, as well as the lateral dispersal of encysted zoospores in still water. This chapter also integrates information from previous chapters regarding loss of viability and the re-emergence of zoospores from cysts with these dispersal studies.

MATERIALS AND METHODS

Lateral dispersal of encysted zoospores of *Phytophthora parasitica*. *Method of cyst recovery.* During preliminary studies, a 17 x 30-cm plastic box was used to simulate a model pond to determine the best method for obtaining data on lateral dispersal of encysted zoospores of *P. parasitica* (isolate GLN 9-3). Zoospore cysts of *P. parasitica* were produced as described previously, and the concentration of zoospores was estimated using a hemacytometer. Inoculum was added to the boxes by pipetting 5 ml of the encysted zoospore suspension with a concentration of 10^5 zoospore cysts/ml in the center of the water body just above the surface.

Three methods were tested to determine the best technique to assess lateral dispersal. For the first method, empty 60 x 15-cm petri plates were placed on the bottom of a plastic box, and the contents that were collected in these plates were viewed microscopically after a designated time interval had passed. For the second method, plates containing P_{10}VPH selective media were placed on the bottom of a box. After the allotted amount of time had passed, the plates were placed upside down in the hood to allow excess water to drain, incubated at 24°C for 24 h, after which colony forming units were counted. For the third method, empty plates were

placed on the bottom of a box and molten P_{10}VPH media (cooled to 48°C to avoid damage to propagules) was added to each plate after the plates were removed. The plates were set aside to allow the media to solidify, incubated at 24°C for 24 h and colony forming units were then counted. For all three methods, plates were turned perpendicular to the bottom of the box before removing carefully. This was done so that no additional zoospores were picked up on the plates during removal.

Method of adding inoculum. Having ascertained that 60 x 15-cm petri plates containing P_{10}VPH media was the best method for cyst recovery, larger boxes (59 x 43 x 30.5 cm) were used to assess lateral dispersal of encysted zoospores when inoculum was added using three different methods. Three identical boxes were used to test the three different methods of adding inoculum. The boxes were filled with ROW to a depth of 10 cm, and plates containing solidified P_{10}VPH media were placed on the bottom before the inoculum was added (Fig. 4.1). Encysted zoospores were produced as described previously and this inoculum was added to the boxes by pipetting 3 ml of the encysted zoospore suspension with a concentration of 10^5 zoospore cysts/ml in the center above the water surface for the first test, in the center approximately 1 cm below the surface of the water for the second test, and at one end of the box approximately 1 cm below the surface of the water for the third test (Fig. 4.2).

Dispersal of dye in model pond. Three ml of blue dye was added to one end of a large box in the same manner as the inoculum in order to visually observe the dispersion of the dye in the model pond.

Dispersal of zoospore cysts in model pond. Once a method was established in the larger boxes, the experiment was repeated using three boxes each time, and lids were placed on top to prevent disturbance. Another experiment was performed using two large boxes and more petri plates added towards the point of inoculation, which was at one end of the box. This experiment produced no improvement in data.

Settling rates of encysted zoospores of *Phytophthora parasitica*. *Settling in glass cylinder.* For preliminary settling experiments, encysted zoospores of *P. parasitica* (isolate

GLN 9-3) were produced and counted as previously described. One ml of the encysted zoospore suspension with a concentration of 10^5 zoospore cysts/ml was added to the top of a 100-ml graduated glass cylinder containing 100 ml SDW. Samples were taken at the 80-ml, 50-ml, and 0-ml marks using a 1-ml pipette inserted gently down along the inside of the cylinder. Samples were plated onto P_{10}VPH selective media and the plates were swirled gently to evenly distribute the sample over the surface. Samples were taken at 0, 30, 60, 120, and 180 min after initiation. A small sample was also taken at each time and at each level to test the use of a hemacytometer to count cysts.

A second series of experiments using graduated glass cylinders focused on settling during the first 60 min. Samples were taken from the top (surface), middle (50-ml mark), and bottom (0-ml mark). Sample times were 0, 10, 20, 30, and 60 min after initiation and samples were assayed as above.

Settling in PVC columns. Series I. Larger columns (65 cm in height) were built from PVC pipe. Teflon/rubber 11-mm aluminum crimp top seals (PGC Scientifics, Frederick, MD) were placed in a row every 15 cm from the bottom (Fig. 4.3). Three-cc syringes with 21-guage needles were used to remove samples through these seals. In this manner samples could be removed with minimal disturbance of the water column. Samples were plated on P_{10}VPH medium to assay for colony forming units as previously described. Two identical columns of PVC pipe were used to determine the best method of adding the inoculum. For the first test, the inoculum was added to the top of the column as previously for the graduated cylinders. For the second test, the inoculum was added to the column, and the column was inverted twice to distribute cysts throughout the column. The columns were sampled after 0, 10, 20, 30, and 60 min at sample levels of 0, 15, 30, 45, and 60 cm from top to bottom. Series II. For this series of measuring settling in PVC columns, inoculum was diluted in the final volume of water needed to fill the column in a 1-L glass Pyrex bottle, mixed thoroughly, and then used to fill the PVC column. This was done to assume that a uniform distribution of the cysts was achieved. This is the same method for determining the settling rate of soil particles in water (Haman et al, 1989).

The sampling levels were top (60 cm), middle (30 cm), and bottom (0 cm) at 0, 30, 60, 90, and 120 min after initiation with three replications per time per height. This was replicated three times with sampling times at 0, 4, 8, 24, 48, and 72 h. In later experiments, the 48 and 72 h sampling times were dropped.

RESULTS

Lateral dispersal of encysted zoospores of *Phytophthora parasitica*. *Method of cyst recovery.* Plates containing P_{10}VPH selective media placed on the bottom of the box was the best method to obtain counts of colony forming units needed to assess lateral dispersal. However, many plates near the point of inoculation had too many colonies to count, and future experiments reduced the concentration of added inoculum taking into account the amount of suspension added and the size of the box.

Method of adding inoculum. The best method for adding inoculum to achieve a maximum dispersal pattern was adding inoculum at one end of the box.

Dispersion of dye in model pond. Fig. 4.4 shows the dispersal pattern of the dye approximately 5 minutes after adding to the box. The dye moved approximately one-fourth the length of the box within 5 minutes.

Dispersal of zoospore cysts in model pond. The dispersal pattern of encysted zoospores of *Phytophthora parasitica* added at one end of a model pond is shown in Fig. 4.5. This graphic representation shows that cysts settled to the bottom (10-cm depth) before uniform lateral dispersal was achieved. It was found that settling was a major factor in the simulated ponds and that lateral dispersal in still water was more limited than anticipated. The settling rate of zoospore cysts was 10 cm in less than 3 h, whereas lateral dispersal showed that 20 to 35% of zoospore cysts moved 10 cm in less than 3 h.

Settling rates of encysted zoospores of *Phytophthora parasitica*. *Settling in glass cylinders.* The method of testing settling in glass cylinders caused too much disturbance and the distance to determine a settling rate was not long enough. The hemacytometer method of enumerating zoospore cysts was not as efficient as using selective media.

Settling in PVC columns. Settling rates of encysted zoospores of *Phytophthora parasitica* in three separate experiments (Experiments A, B, and C) in 65-cm water columns are shown in Fig. 4.6, 4.7, and 4.8, respectively. Mean colony forming units after 24 h in samples taken at 60 cm, 30 cm, and 0 cm were 103.3, 22.7, and 14.7 respectively. The settling rate was found to be 60 cm in 24 h. There was also an increase in the proportion in zoospores recovered at 8 h in the upper portion of the PVC column.

DISCUSSION

The settling studies show that encysted zoospores settled 60 cm within the first 24 h. However, re-emergence and loss of viability also played an important role in the settling columns. During the first 24 h, viability declined rapidly as shown in Chapter III. This combined with settling to eliminate cysts from the upper parts of the column. The cyst derived zoospores which re-emerged after 8 h also complicated this interpretation. Although a certain percentage of motile zoospores were temporarily motile at the upper surface, these encysted and succumbed as the original cysts did. Many zoospores could theoretically re-emerge and move to the water surface, but the loss of viability is likely to prevent this from overwhelming the pattern. These factors are all taking place in the column while settling is also occurring. The studies of lateral dispersal of encysted zoospores of *Phytophthora parasitica* demonstrate that lateral dispersion in still water in model ponds under laboratory conditions affects overall dispersal less than settling.

These results demonstrate facets of the physical dispersal of encysted zoospores in water. The research suggests that longer retention times not only allow natural declines in viability, but also allow settling out of encysted zoospores within 24 h. Because encysted zoospores settle to the bottom and a proportion of motile zoospores are found at the surface, management strategies should include drawing water from the middle of retention basins to minimize concentrations of pathogens being recycled.

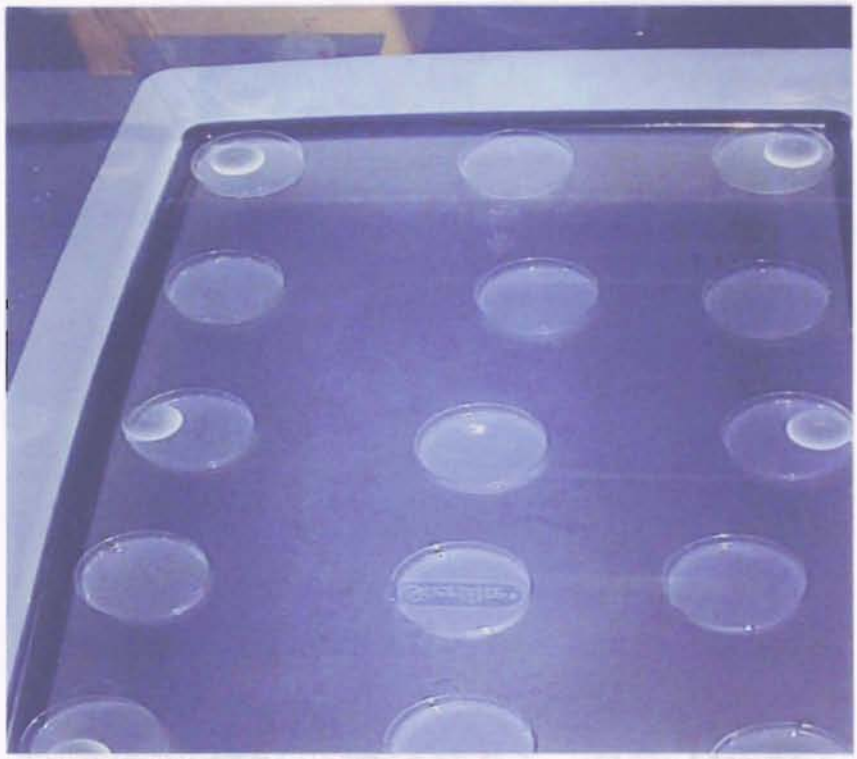

Fig. 4.1 Method of cyst recovery for lateral dispersal studies.

Fig. 4.2 Method of adding inoculum to model ponds for lateral dispersal studies.

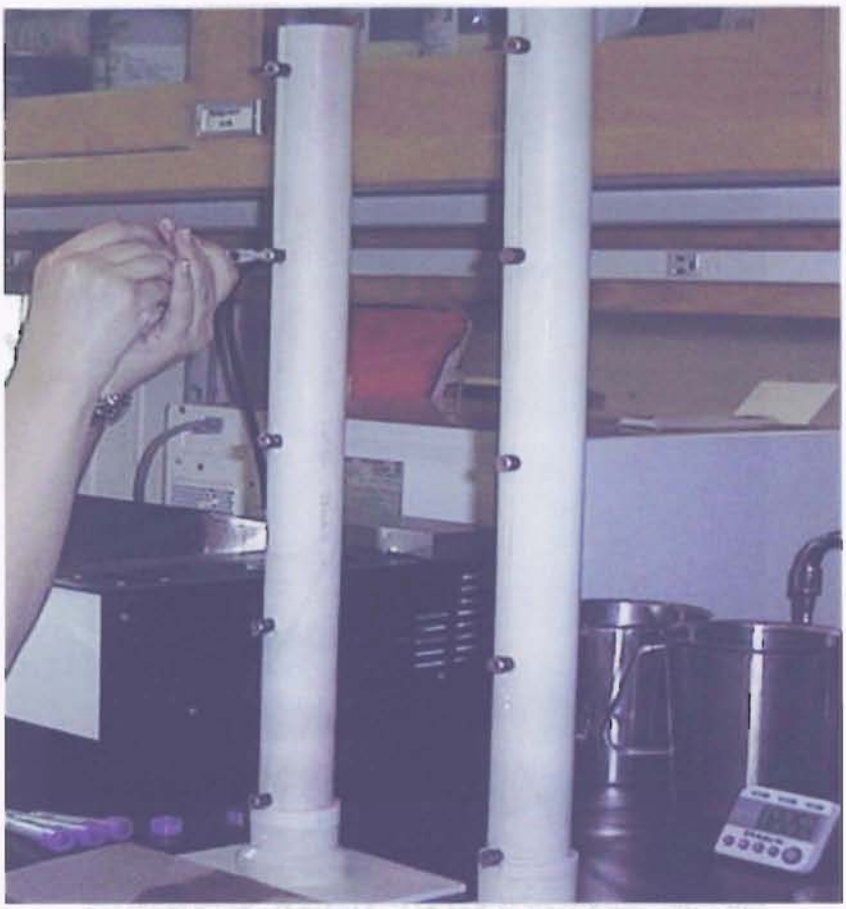

Fig. 4.3 Settling columns (65 cm in height) built of PVC with seals at 15 cm intervals for removing samples.

Fig. 4.4 Image of dye in model ponds after adding on one end of box in same manner as zoospore suspension after approximately 5 minutes.

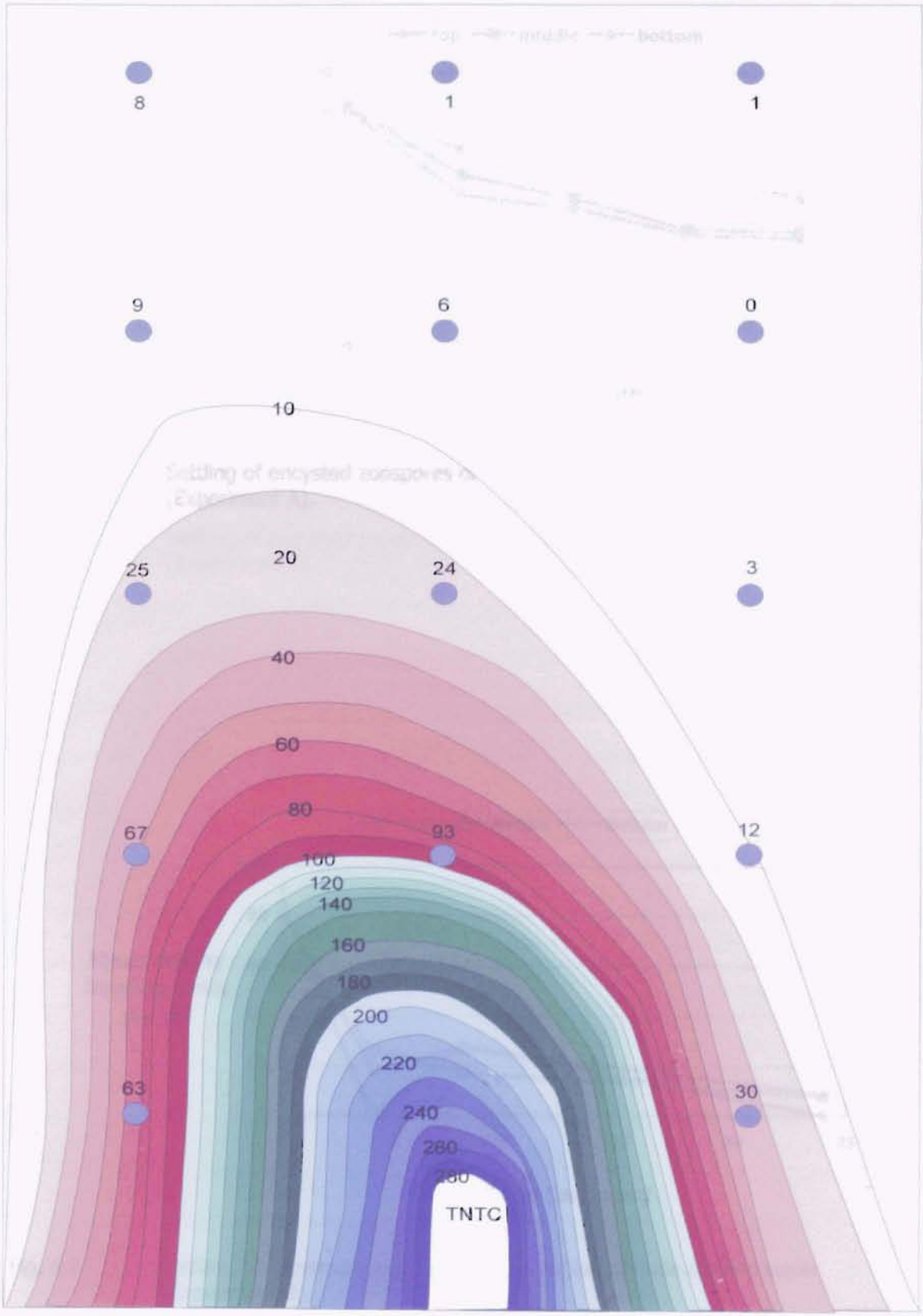

Fig. 4.5 Lateral dispersal of encysted zoospores of *Phytophthora parasitica* in model pond after 3 hours.

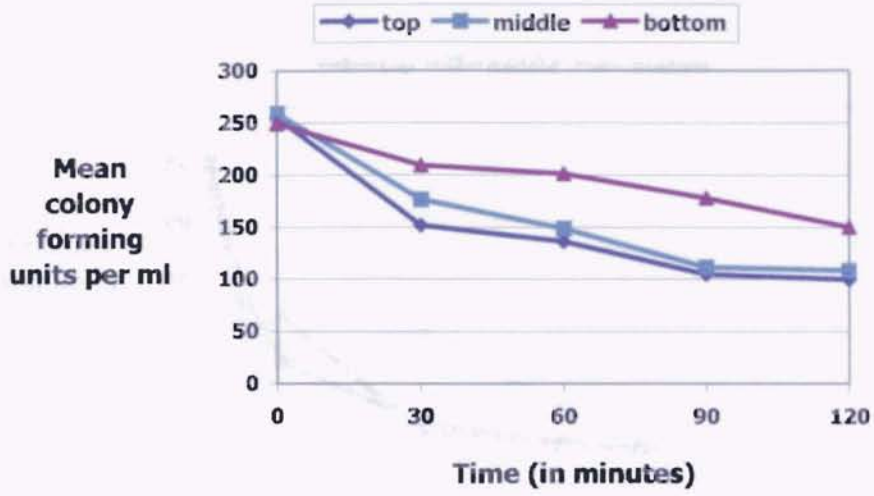

Fig. 4.6 Settling of encysted zoospores of *P. parasitica* in sterile distilled water (Experiment A).

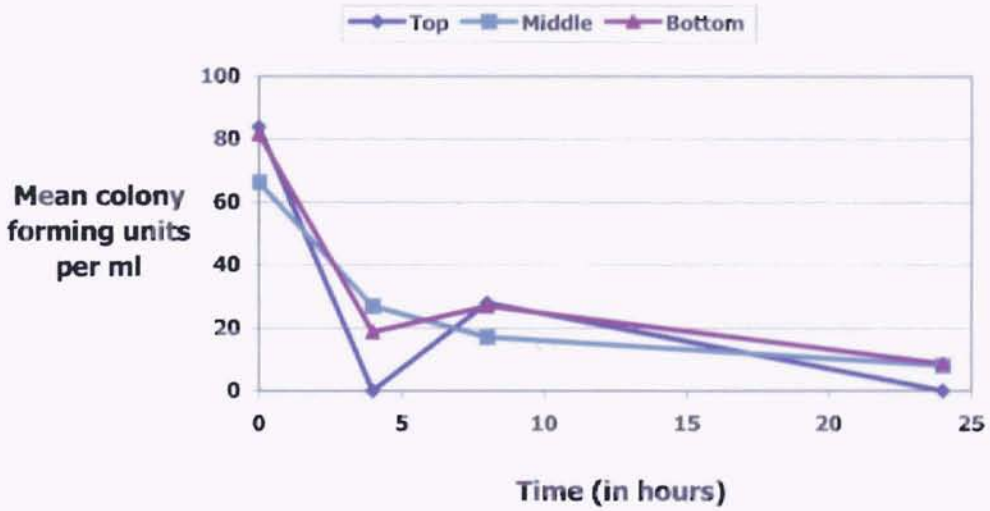

Fig. 4.7 Settling of encysted zoospores of *P. parasitica* in sterile distilled water (Experiment B).

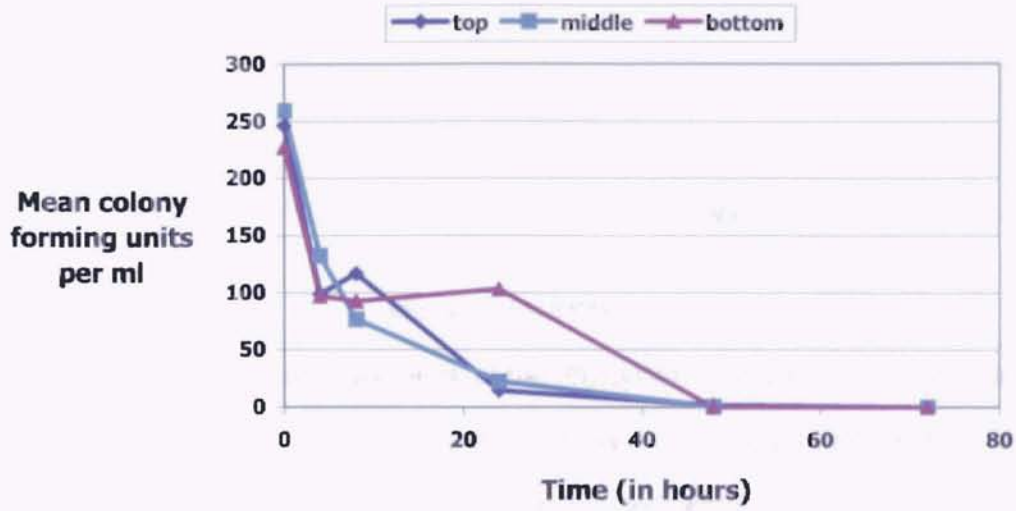

Fig. 4.8 Settling of encysted zoospores of *P. parasitica* in sterile distilled water (Experiment C).

CHAPTER V

SUMMARY AND RECOMMENDATIONS

RESEARCH SUMMARY

Zoospores comprised greater than 94% of the *Phytophthora* propagules recovered in runoff entering retention basins at Greenleaf Nursery Company over the growing season. For this reason zoospores were the principal propagule used in experimental studies.

Release of zoospores and modes of germination by *Phytophthora* spp. in different lake waters was investigated because these processes directly relate to what is taking place in retention basins. Water source and water type did not affect zoospore production or germination. Repeated emergence was predominant over direct germination by germ tube in lake waters. A significant finding of this research was the recording of multiple generations, or repeated emergence, in *Phytophthora* spp. Three generations were scored with a fourth generation observed. This suggests that multiple generations of zoospores are produced in basins. These cyst-derived zoospores were found to be as pathologically competent as sporangial derived zoospores as shown in the study comparing the infection of vinca seedlings. There was no significant difference between the two types of zoospores in their potential to infect.

Decline in viability under laboratory conditions was not affected by water source or water quality with respect to the presence of microorganisms. Survival rates declined rapidly during the first 48 h and were very similar across all types of water and for different *Phytophthora* spp. Viability under field conditions demonstrated that rates of decline during an exposure period were consistent throughout the season, establishing that rapid loss of viability occurred in the first 24 to 48 h as predicted by laboratory studies.

Settling rates were measured in still water in PVC columns. Seventy-four percent of zoospore cysts settled 60 cm (water column height) within 24 h. Settling occurred more quickly than lateral dispersal in still water under laboratory conditions.

RECOMMENDATIONS TO THE NURSERY INDUSTRY

Recycling nursery irrigation water affects programs and disease management strategies. Routine scouting for early detection of disease is an important aspect of any disease management program. Removal of diseased plants to prevent spread of pathogens into drainage water is an essential part of scouting in order to reduce levels of pathogens in the irrigation system.

Irrigation water in different parts of a recycling irrigation system will be of different quality with respect to plant pathogens. Careful irrigation management is needed to make sure that the pathogen status of each type of water matches the susceptibility of the plant materials receiving that water. Crops that are highly susceptible to water-borne pathogens such as *Phytophthora* spp. should be grouped together in the same part of the nursery to permit more efficient irrigation management. Recycled water should be used for hardy plants, fresh or disinfested recycled water should be used for sensitive plants, and pathogen-free water should be used for propagation.

Captured runoff should be stored and mixed in ways that promote settling and dilution of pathogens with fresh water or storm water. Retention times in basins should be maximized so that pathogens are reduced in recycled irrigation water. Natural reductions in viability of pathogens and settling can reduce pathogen levels. Water should be drawn from the middle of retention basins since encysted zoospores settle to the bottom, while motile zoospores swim to the top. Water should also be drawn from a point farthest from the inlet into a retention basin to optimize retention time.

Monitoring for *Phytophthora* spp. in irrigation water should be carried out periodically. Water should be sampled at various points in the irrigation system, since pathogens may be problematic

only in certain parts of the system. Evaluating the level of pathogens in irrigation water will help determine the quality of water to be recycled.

SELECTED BIBLOGRAPHY

Ainsworth, G.C. 1961. A dictionary of the fungi. Commonwealth Mycological Institute, Kew, Surrey.

Blackwell, E. 1949. Terminology in *Phytophthora*. Mycological Paper No. 30. Commonwealth Mycological Institute, Kew, Surrey.

Fain, G.B., Gilliam, C.H., and Tilt, K.M. 1998. Runoff nitrogen levels from two Alabama nurseries. SNA Research Conference 43:510-512.

Gregory, P.H. 1973. The microbiology of the atmosphere. Halsted Press.

Haman, D.Z., Smajstrla, A.G., and Zazueta, F.S. 1989. Settling basins for trickle irrigation in Florida. Univiversity of Florida Extension Service: 5 pp.

Ho, H.H., and Hickman, C.J. 1967. Asexual reproduction and behavior of zoospores of *Phytophthora megasperma* var. sojae. Canadian Journal of Botany 45:1963-1981.

Jeffers, S.N., and Wilcox, W.F. 1990. *Phytophthora* crown, collar, and root rots. Pages 43-45 in: A Compendium of Apple and Pear Diseases. The American Phytopathological Society, St. Paul, MN.

Lovell, C.J., and Rose, C.W. 1988. Measurement of soil aggregate settling velocities. I. A modified bottom withdrawal tube method. Australian Journal of Soil Research. 26:55-71.

Lynch, J.M., and Hobbie, J.E. 1988. Micro-organisms in action: concepts and applications in microbial ecology. Blackwell Scientific Publications.

MacDonald, J.D., Lagunas-Solar, M., and Kabashima, J. 1994a. Research update: treatment of irrigation effluent water to eliminate plant pathogens. California Nurserymen 46-48.

MacDonald, J.D., Ali-Shtayeh, M.S., Kabashima, J., and Stites, J. 1994b. Occurrence of *Phytophthora* species in recirculated nursery irrigation effluents. Plant Disease 78:607-611.

Matheron, M.E., and Porchas, M. 1996. Colonization of citrus roots by *Phytophthora citrophthora* and *P. parasitica* in daily soil temperature fluctuations between favorable and inhibitory levels. Plant Disease 80:1135-1140.

Matheron, M.E., Porchas, M., and Matejka, J.C. 1997. Distribution and seasonal population dynamics of *Phytophthora citrophthora* and *P. parasitica* in Arizona citrus orchards and effect of fungicides on tree health. Plant Disease 81:1384-1390.

Mircetich, S.M., and Matheron, M.E. 1976. *Phytophthora* root and crown rot of cherry trees. Phytopathology 66:549-558.

McIntosh, D.L. 1966. The occurrence of *Phytophthora spp.* in irrigation systems in British Columbia. Canadian Journal of Botany 44:1591-1596.

Neher, D. and Duniway, J.M. 1992. Dispersal of *Phytophthora parasitica* in tomato fields by furrow irrigation. Plant Disease 76:582-586.

Ribeiro, O.K. 1978. A source book of the genus *Phytophthora*. Germany: Strauss and Cramer.

Salvin, S.B. 1940. The occurrence of five successive swarming stages in a non-sexual Achlya. Mycologia 32:148-154.

Shokes, F.M., and McCarter, S.M. 1979. Occurrence, dissemination, and survival of plant pathogens in surface irrigation ponds in southern Georgia. Phytopathology 69:510-516.

Smither-Kopperl, M.L., Charudattan, R., and Berger, R.D. 1998. Dispersal of spores of *Fusarium culmorum* in aquatic systems. Phytopathology 88:382-388.

Steadman, J.R., Maier, C.R., Schwartz, H.F., and Kerr, E.D. 1975. Pollution of surface irrigation waters by plant pathogenic organisms. Water Resources Bulletin 11:796-804.

Thomson, S.V., and Allen, R.M. 1976. Mechanisms of survival of zoospores of *Phytophthora parasitica* in irrigation water. Phytopathology 66:1198-1202.

United States Environmental Protection Agency. 1992. Managing containerized nurseries for pollution prevention. Dallas, Texas: Water Management Division, Region VI:6pp.

von Broembsen, S.L., and Deacon, J.W. 1996. Effects of calcium on germination and further release from zoospore cysts of *Phytophthora parasitica*. Mycological Research 100:1498-1504.

von Broembsen, S.L., and Deacon, J.W. 1997. Calcium interference with zoospore biology and infectivity of *Phytophthora parasitica* in nutrient irrigation solutions. Phytopathology 87:522-528.

Wilson, S.K. 1998. Managing the plant pathogen, *Phytophthora*, to improve acceptance of recycling technology in ornamental nurseries. MS thesis, Oklahoma State University, December 1998.

Wright, R.D. 1988. Nursery runoff: a future challenge. The International Plant Propagators' Society 38:417-419.

VITA

Nikki D. Charlton

Candidate for the Degree of

Master of Science

Thesis: EPIDEMIOLOGY AND MANAGEMENT OF *PHYTOPHTHORA* SPP. IN RUNOFF FROM ORNAMENTAL NURSERIES USING RECYCLING IRRIGATION SYSTEMS

Major Field: Environmental Science

Biographical:

Personal Data: Born in Oklahoma City, Oklahoma, on September 14, 1976, the daughter of Ron and Jeanette Charlton.

Education: Graduated from Casady School, Oklahoma City, Oklahoma in June 1995; received Bachelor of Science degree in Biological Sciences from Oklahoma State University, Stillwater, Oklahoma in May 1999. Completed the requirements for the Master of Science degree with a major in Environmental Science at Oklahoma State University in May 2001.

Experience: Employed by Oklahoma State University as a laboratory technician in the Department of Entomology and Plant Pathology as an undergraduate and as a graduate research assistant; Oklahoma State University, Department of Entomology and Plant Pathology, 1998 to present.

Professional Membership: American Phytopathological Society