

**FILTRATION OF PHYTOPHTHORA AND PYTHIUM ZOOSPORES
IN IRRIGATION WATER**

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ABSTRACT

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Phytophthora and *Pythium* are commonly known as water molds, and can cause enormous damages to many floriculture and vegetable crops worldwide, including seedling damping-off, stunting, and crown, stem and root rot. It is challenging to control these pathogens because plants can be infected and do not show symptoms until the disease is too advanced to respond to treatment. The pathogens can also easily develop resistance to effective fungicides. As the zoospore movement with water flow is a major transmission pathway of these pathogens, understanding the transport of zoospores in natural and engineered systems is critical to developing strategies to control the pathogens in both field and greenhouse crops. Thus, the first study investigated the transport and retention of *Phytophthora capsici* zoospores in saturated columns packed with iron oxide coated sand (IOCS) or uncoated sand in Na⁺ or Ca²⁺ background solution at pH 7.2 or 4.4, in combination with XDLVO interaction energy calculations and microscopic visualizations. Significantly more encysted zoospores were retained in IOCS than in uncoated sand, and at pH 4.4 than at pH 7.2, which likely resulted from increased electrostatic attraction between zoospores and grain surface. At pH 7.2, up to 99% and 96% of the encysted zoospores were removed in IOCS and uncoated sand, respectively, due to a combination of strong surface attachment, pore straining, and adhesive interactions. Motile biflagellate zoospores were more readily transported than encysted zoospores, thus posing a greater dispersal and infection risk.

The second and third studies were conducted in a greenhouse to demonstrate a proof-of-concept of using fast-flow filtration to control *Phytophthora* and *Pythium* diseases in greenhouse floriculture and vegetable crops. The second study showed that *Pythium aphanidermatum* could be effectively removed by the fast-flow sand and AC filters at low water pressure. The rapid sand filter had the best performance because no decrease in the poinsettia quality was observed when compared to the non-inoculated control plants. Because the AC filter could also remove the essential nutrients from the irrigation water, and cause the Fe deficiency in the poinsettias, it is less desirable to be used unless the nutrients can be supplied separately instead of through irrigation water. The third study found that the filter with iron oxide coated media [IOCM] could effectively protect the squash plants from *Phytophthora capsici*, but caused the nutrient deficiency in the squash. The sand filter could not prevent, but only slow the disease development in the squash. Again, it shows that the IOCM filter has the potential to be used in treating irrigation water in the greenhouse vegetable production, but sufficient nutrients also need to be provided. Overall, the results suggested that physical removal of pathogens using fast-flow filtration can overcome many limitations of fungicide application, and may be a promising alternative for disease management in greenhouses.

This thesis is dedicated to my parents, Hyeongu Jeon and Oknam Choi, my wife
Yiseul Kim, and my daughter Yuna K. Jeon

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CHAPTER 1

1.1. INTRODUCTION

Phytophthora and *Pythium*, also known as “water molds”, are a group of the most notorious plant pathogens. *Phytophthora* and *Pythium* are fungus-like organisms that cause seedling damping-off, stunting, and stem, crown, and root rot diseases in both floriculture and vegetable crops, thus limiting their production and resulting in devastating crop losses.^{1, 2} These pathogens are ubiquitous in the environment and infect a wide range of greenhouse floral and vegetable crops. The water molds are challenging to control because plants can be infected and do not show symptoms until the disease is too developed to respond to any treatments.³ Moreover, they can easily adjust to the environment especially under warm and wet conditions in a greenhouse. Thick-walled oospores can survive for several years on plant containers, benches, floors, and in potting media or soils. Furthermore, there are a limited number of fungicides effective against these water molds, and pathogen resistance to effective fungicides is a primary concern. These zoosporic organisms are generally found and well suited to the aquatic environment for the movement and dissemination of their disease-inciting swimming spores (i.e., zoospores).⁴ Water is the primary factor to release and transfer zoospores from sporangia of *Phytophthora* and *Pythium* to a plant host. The disease epidemics are mainly attributed to the rapid dispersal of the zoospores by flowing water during rainfall and irrigation events, and easy recognition of a host tissue by either autotaxis, chemotaxis, or electrotaxis. The movement of *Phytophthora* and *Pythium* zoospores in the environment is dependent on their surface properties (including surface charges, hydrophobicity, and bio-adhesive secretion), surface properties of porous media (e.g.,

surface charges, and grain size), as well as solution chemistry of the carrying water (e.g., solution pH, and ionic strength and composition). Understanding the zoospore movement in porous media is not only important to the transport and retention of the zoospores in soil profiles, but also to developing effective engineered filtration systems for the pathogen removal.

In a greenhouse setting, the management of *Phytophthora* and *Pythium* is particularly challenging, especially when irrigation water is recycled. Recycling irrigation water including nutrients is beneficial both from environmental and economical perspectives.⁴ Thus, irrigation water recycling in the greenhouses is becoming increasingly popular. Meador et al.⁵ reported that 50% of the 24 surveyed greenhouses in the U.S. recycled irrigation water, and the largest water users recycled 75–100% of their irrigation water (490,000–1,000,000 gallons per day). However, this beneficial practice can aggravate the transmission of water mold pathogens^{6,7} as their spread occurs when the motile and encysted zoospores move with the flowing water. They may be introduced to floriculture or vegetable crops in greenhouses via many ways including plant plugs or other prefinished plant material. Or it can hibernate on dirty plant containers, benches, and walkways or even ventilation systems. Hong and Moorman⁴ reported that 16 *Phytophthora* spp. and 26 *Pythium* spp. were isolated from nursery and greenhouse operations. Moreover, a survey conducted by Michigan State University (MSU) showed that several *Pythium* spp. were identified from a wide range of floriculture crops. Common *Pythium* spp. recovered from geranium were *P. irregulare* (68%) and *P. aphanidermatum* (22%). Nearly all (96%) of the *Pythium* spp. recovered from snapdragon were *P. irregulare*. *Pythium* spp. isolated from hibiscus included *P.*

irregulare (50%) and *P. segnitium* (50%). *P. irregulare* infects most lantana (83%). The most prevalent *Pythium* spp. isolated from poinsettia included *P. ultimum* (53%) and *P. aphanidermatum*. More importantly, the cycle of plant infection and zoospore production is very short through asexual reproduction.^{8,9} Therefore, even low levels of *Phytophthora* and *Pythium* in recycled irrigation water can result in a rapid epidemic if the irrigation water is not properly treated.

Various chemical and physical methods have been proposed to disinfect the contaminated irrigation water in greenhouses, including filtration, chlorination, copper ionization, ozonation, UV light, activated peroxygens, chlorine dioxide, and heat.^{4, 10} Advantages, disadvantages, and characteristics of these methods are summarized in Table 1.1. Chemical treatments are known to introduce resistance among pathogen populations, which eventually make these treatments less effective.¹¹ Moreover, a high level of chemical dose may cause phytotoxicity to crops.¹² Therefore, filtration is an appealing cost-effective treatment to remove *Pythium* and *Phytophthora* from contaminated water.^{4, 13, 14} Currently, screen or disk filters with a pore size > 100 µm are used to remove large particles such as potting soil particles and plant debris to avoid clogging of the drippers.^{5, 10} Also, these filters with large pore size are used as pre-treatment to improve the efficiency of other disinfection methods such as heat treatment, ozone treatment, or UV radiation.¹⁰ Membrane filters of relatively small pore size (<5µm, micro-filtration) can be used to remove the water mold pathogens, but requires relatively high water pressure and high maintenance cost due to clogging and leaking problems.¹⁵ Slow sand filtration has been used for drinking water treatment, and also investigated for the removal of water molds in the greenhouse applications.^{13, 16-18} However, it has not been used in commercial

greenhouse.¹² Slow sand filters are easy to operate and maintain. Water flows slowly through a packed bed of granular sand media at a rate of 100–300 L/m²/h.^{12, 15} However, the water flow is too low to meet the water demand in a typical greenhouse, which severely limits the wide adoption of filtration technique. Additionally, variable performance has been observed with sand filtration systems.^{14, 19} Clearly, additional research on fast-flow filtration is needed to first show the proof-of-concept of using filtration systems in controlling the plant pathogens in the greenhouses. Better design of filtration systems is often dependent on fundamental mechanisms controlling the pathogen transport and retention in porous media.

1.2. STUDY OBJECTIVES

In light of the above overview, the objectives of this work were to the following

I. Investigate the transport and retention of *Phytophthora capsici* zoospores in saturated sand columns as influenced by environmental factors such as sand grain size, iron oxide grain coating, solution pH, and cation type. The mobility of biflagellate and encysted zoospores was also compared to elucidate the role of encystment in the zoospore transport.

II. Investigate the effectiveness of fast-flow filtration to control *Pythium* root rot in potted poinsettias in greenhouses using the ebb-and-flow and flood-floor irrigation systems. Two greenhouse experiments were performed to investigate the effect of filter media type (i.e., sand and activated carbon), fungicide application (i.e, etridiazole), and pathogen transmission mode (i.e., inoculation in plants vs in irrigation water).

III. Investigate the effectiveness of fast-flow filtration to control *Phytophthora* diseases for potted squashes in greenhouses using the ebb-and-flow and flood-floor irrigation systems. Two greenhouse experiments were carried out to test the effect of filter media type (i.e., sand and iron oxide coated media [IOCM]) and fungicide application (i.e, etridiazole).

The following chapters address the three objectives of my research. The Objective I is addressed in Chapter 2, Objective II in Chapter 3, and Objective III in Chapter 4. The dissertation ends with Chapter 5 that summarizes the findings of the studies that have been conducted and identifies future research directions.

Table 1.1. Currently available disinfestation methods for recirculation systems and their advantages, disadvantages, and characteristics.

Method of disinfestation	Advantages	Disadvantages	Characteristics
Non-Chemical Methods			
1. Heat treatment	Highly effective	very energy intensive	temperature setpoint (95°C) exposure time 10s
2. UV Radiation	Low space requirement	Efficiency drops with high organic matter and bulb age Interaction with micronutrients	Wavelengths between 200 and 280 nm
3. Filtration			
a. Screen filtration	Low cost	No pathogen removal	Remove large particles Pre-treatment for heat, ozone, and UV radiation
b. Membrane filtration	Highly effective	Frequent plugging and leaks High capital costs	Size based filtration
c. Slow sand filtration	Low cost	High space requirement Effectiveness varies with pathogen Low flow rate	Bio-film
Chemical methods			
1. Ozone (O ₃)	Highly effective	High capital costs High maintenance costs Efficiency drops with high organic matter Reacts with iron chelate	Not popular because of harmful for human and strict rules
2. Hydrogen peroxide (H ₂ O ₂)	Low cost	not efficient	Different dosages are recommended
3. Chlorine	Low cost	Phytotoxicity at high concentrations	Strong oxidation including Sodium hypochlorite(NaOCl), Chlorine Dioxide (ClO ₂), and sodium hypochlorite
4. Fungicide	Low cost	Phytotoxicity at high concentrations Few fungicides available for water mold pathogen Pathogen quickly develop resistance to the effective fungicides	

REFERENCES

REFERENCES

1. Granke, L. L.; Quesada-Ocampo, L.; Lamour, K.; Hausbeck, M. K., Advances in Research on *Phytophthora capsici* on Vegetable Crops in The United States. *Plant Dis* **2012**, 96, (11), 1588-1600.
2. Themann, K.; Werres, S.; Luttmann, R.; Diener, H. A., Observations of *Phytophthora* spp. in water recirculation systems in commercial hardy ornamental nursery stock. *Eur J Plant Pathol* **2002**, 108, (4), 337-343.
3. Olson, H. A.; Benson, D. M., Characterization of *Phytophthora* spp. on Floriculture Crops in North Carolina. *Plant Dis* **2011**, 95, (8), 1013-1020.
4. Hong, C. X.; Moorman, G. W., Plant pathogens in irrigation water: Challenges and opportunities. *Crit. Rev. Plant Sci.* **2005**, 24, (3), 189-208.
5. Meador et al., Survey of physical, chemical, and microbial water quality in greenhouse and nursery irrigation water. *HortTechnology* **2012**, 22(6), 778-786.
6. Hong, C. X.; Richardson, P. A.; Kong, P.; Bush, E. A., Efficacy of chlorine on multiple species of *Phytophthora* in recycled nursery irrigation water. *Plant Dis* **2003**, 87, (10), 1183-1189.
7. Stanghellini, M. E.; Rasmussen, S. L., Identification and Origin of Plant-Pathogenic Microorganisms in Recirculating Nutrient Solutions. *Adv Space Res* **1994**, 14, (11), 349-355.
8. Gevens, A.; Donahoo, R.; Lamour, K.; Hausbeck, M., Characterization of *Phytophthora capsici* from Michigan surface irrigation water. *Phytopathology* **2007**, 97, (4), 421-428.
9. Erwin, D. C.; Ribeiro, O. K., *Phytophthora diseases worldwide*. American Phytopathological Society (APS Press): 1996.
10. van Os, E. A., Comparison of Some Chemical and Non-Chemical Treatments to Disinfect a Recirculating Nutrient Solution. *International Symposium on Soilless Culture and Hydroponics* **2009**, 843, 229-234.
11. Lamour, K. H.; Daughtrey, M. L.; Benson, D. M.; Hwang, J.; Hausbeck, M. K., Etiology of *Phytophthora drechsleri* and *P. nicotianae* (= *P. parasitica*) diseases affecting floriculture crops. *Plant Dis.* **2003**, 87, (7), 854-858.
12. Raudales, R. E.; Parke, J. L.; Guy, C. L.; Fisher, P. R., Control of waterborne microbes in irrigation: A review. *Agr Water Manage* **2014**, 143, 9-28.

13. Calvo-Bado, L. A.; Pettitt, T. R.; Parsons, N.; Petch, G. M.; Morgan, J. A. W.; Whipps, J. M., Spatial and temporal analysis of the microbial community in slow sand filters used for treating horticultural irrigation water. *Applied and environmental microbiology* **2003**, 69, (4), 2116-2125.
14. Martínez, F.; Castillo, S.; Carmona, E.; Avilés, M., Dissemination of *Phytophthora cactorum*, cause of crown rot in strawberry, in open and closed soilless growing systems and the potential for control using slow sand filtration. *Scientia horticultrae* **2010**, 125, (4), 756-760.
15. Ehret, D. L.; Alsanius, B.; Wohanka, W.; Menzies, J. G.; Utkhede, R., Disinfestation of recirculating nutrient solutions in greenhouse horticulture. *Agronomie* **2001**, 21, (4), 323-339.
16. Lee, E.; Oki, L. R., Slow sand filters effectively reduce *Phytophthora* after a pathogen switch from *Fusarium* and a simulated pump failure. *Water Res* **2013**, 47, (14), 5121-5129.
17. Ufer, T., Werres, S. K., Posner, M., and Wessels, H.-P., Filtration to eliminate *Phytophthora* spp. from recirculating water systems in commercial nurseries. *Online Plant Health Progress* **2008**, <http://dx.doi.org/10.1094/PHP-2008-0314-01-RS>.
18. van Os, E. A.; Amsing, J. J.; van Kuik, A. J.; Willers, H., Slow sand filtration: A potential method for the elimination of pathogens and nematodes in recirculating nutrient solutions from glasshouse-grown crops. *International Symposium on Growing Media and Hydroponics, Vols I and II* **1998**, (481), 519-526.
19. Déniel, F.; Rey, P.; Chérif, M.; Guillou, A.; Tirilly, Y., Indigenous bacteria with antagonistic and plant-growth-promoting activities improve slow-filtration efficiency in soilless cultivation. *Canadian journal of microbiology* **2004**, 50, (7), 499-508.

CHAPTER 2

TRANSPORT AND RETENTION OF PHYTOPHTHORA CAPSICI ZOOSPORES IN SATURATED POROUS MEDIA

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ABSTRACT

Phytophthora capsici is an important plant pathogen capable of infecting several major vegetable crops. Water-induced *P. capsici* transport is considered to be a significant contributor to disease outbreaks and subsequent crop loss. However, little is known about factors controlling *P. capsici* zoospore transport in porous media, thus impeding my understanding of their environmental dispersal and development of filtration techniques for contaminated irrigation water. This study investigated the transport and retention of *P. capsici* zoospores in saturated columns packed with iron oxide coated sand (IOCS) or uncoated sand in Na⁺ or Ca²⁺ background solution at pH 7.2 or 4.4, in combination with XDLVO interaction energy calculations and microscopic visualizations. Significantly more encysted zoospores were retained in IOCS than in uncoated sand, and at pH 4.4 than at pH 7.2, which likely resulted from increased electrostatic attraction between zoospores and grain surface. At pH 7.2, up to 99% and 96% of the encysted zoospores were removed in IOCS and uncoated sand, respectively, due to a combination of strong surface attachment, pore straining, and adhesive interactions. Motile biflagellate zoospores were more readily transported than encysted zoospores, thus posing a greater dispersal and infection risk. This study has broad implications in

environmental transport of *Phytophthora* zoospores in natural soils as well as in cost-effective engineered filtration systems.

2.1. INTRODUCTION

Plant pathogens cause annual crop loss of approximately \$33 billion in the US despite large pesticide use for disease control; subsequent environmental release of used pesticides can also lead to enormous human and ecosystem health costs.^{20, 21} Control of plant pathogens, specifically those that can be disseminated through water flow, requires a better understanding of their transport in porous media such as soils. However, studies on the transport of plant pathogens (e.g., *Phytophthora*) in porous media are surprisingly scarce.²² This is in contrast to extensive work regarding the transport of microbial human pathogens (e.g., bacteria, virus, and protozoa) for the purpose of protecting water quality and human health.²³⁻²⁶ This paucity of research impedes the development of effective measures to mitigate environmental dispersal of plant pathogens. In the era of “one health”, the research on plant pathogen transport is equally important in the context of food security and protection of human and environmental health.

In this study I primarily focused on the oomycete *Phytophthora capsici*, a fungal-like organism that infects many vegetables in the field and greenhouse.^{1, 4, 27, 28} Yield loss from *P. capsici* infection was reported to be 40–100% for peppers, 20% for squash, and 65% for greenhouse-grown cucumbers.²⁸ Sometimes total crop loss can occur due to *P. capsici* outbreaks.²⁷ Each year in Michigan alone, up to 25% of the state’s \$134 million vegetable value may be lost due to diseases caused by *P. capsici*.²⁷ Hence, control of *P. capsici* outbreaks is critical to many agricultural producers, and requires an integrated management approach that targets pathogen survival, development and dispersal.^{1, 27, 29} Among recommended mitigation strategies for *P. capsici* (e.g., crop rotation, irrigation management, fungicide treatment, and host-plant resistance), soil water management to

minimize water-induced pathogen dissemination within a field or greenhouse is important to the successful control of this pathogen.^{1, 27, 29} A key feature of the *P. capsici* disease cycle is the production of 20–40 biflagellate swimming spores (i.e., zoospores) from a single non-motile sporangium on infected plant tissue upon exposure to rainfall, irrigation water, or surface runoff.^{1, 27} Each of thousands of released zoospores has the potential to infect a host plant. Thus, the transport of virulent *P. capsici* zoospores promotes plant disease development.^{1, 8, 28} The flow-induced pathogen transmission in soils spreads the pathogen across fields, and the pathogen-laden drainage water could contaminate shallow groundwater or surface water. Irrigation with surface water contaminated with *P. capsici* is an important contributor to disease outbreaks in field-grown vegetable crops.^{1, 8, 28} When contaminated surface water cannot be avoided, treating the water with rapid sand filtration and chlorination has been implemented in the field.³⁰

Similarly, in greenhouses disease outbreaks are often exacerbated by recycling irrigation water.^{4, 31, 32} Irrigation water recycling is widely practiced by many greenhouse growers to reduce pollutant discharge or alleviate water shortage.^{32, 33} For instance, some greenhouses with significant water use (i.e., 0.5–1 million gallons a day) recycle 75–100% of their irrigation water.³⁴ Treatment of recycled irrigation water containing pathogens is common in greenhouses and includes physical, chemical and ecological methods.^{4, 35, 36} Plants are often treated with fungicides to reduce disease, but fungicides are not always efficacious. Sustained fungicide treatment may prompt the development of fungicide resistance within *P. capsici* populations.^{37, 38} High chemical doses can also cause phytotoxicity to some crops³⁵ and pose significant environmental risk. In contrast, physical removal by filtration does not have the abovementioned limitations, and can be

an attractive alternative to control the pathogen transmission in recirculating systems.^{4, 13, 14, 31, 39} Nonetheless, filtration techniques still need to be refined to improve performance and facilitate adoption.³⁶

Hence, control of *P. capsici* dispersal in the field and greenhouse requires mechanistic understanding of factors governing the transport of zoospores in porous media. A limited number of studies revealed that water-induced passive movement of *Phytophthora* zoospores is more effective in transporting them over a greater distance in soils than the autonomous, active movement of zoospore swimming.^{40, 41} Active movement only allows the zoospores to move a maximum of a few millimeters to centimeters,⁴¹⁻⁴³ and collision with solid surface often results in encystment and a subsequent loss in motility.^{41, 44, 45} However, the studies on the passive transport of *Phytophthora* zoospores have been very limited.²² Transport of *Phytophthora* zoospores in saturated porous media may be determined by several well-known colloid retention mechanisms, including attachment at the solid-water interface, straining at the grain-to-grain contacts, and mechanical filtration due to size-constraint at pore throats.²³ Nonetheless, *Phytophthora* zoospores differ from many previously studied biocolloids (e.g., bacteria, virus and protozoa) due to their relatively large size ($\sim 7.5 \mu\text{m}$) and its encystment phase. Encystment could even occur in bulk solution without surface collision. During encystment, zoospores lose their flagella and are transformed from oval shape to spherical shape.^{46, 47} Size and shape of colloids significantly affect their transport behavior,⁴⁸⁻⁵¹ in addition to many environmental factors such as solution chemistry, collector grain size, and surface properties of colloids and collector grains.²³ Therefore, in order to fill the knowledge gap on plant pathogen transport, this study aimed to

investigate the transport and retention of *P. capsici* zoospores in saturated sand columns as influenced by environmental factors including grain size, iron oxide grain coating, solution pH, and cation type. The mobility of biflagellate and encysted zoospores was also compared to elucidate the role of encystment in the zoospore transport. This study combined column transport experiments, microscopic visualization, and surface interaction energy calculations to elucidate retention mechanisms of *P. capsici* zoospores in porous media.

2.2. MATERIALS AND METHODS

2.2.1. Zoospore Suspensions

Phytophthora capsici isolate 11127 used in this study was obtained from the culture collection maintained in the laboratory of M.K. Hausbeck at Michigan State University. Isolate 11127 was originally isolated from a wax bean leaf from a field in Van Buren County near Keeler, MI and was characterized as an A1 mating type and insensitive to the fungicide mefenoxam.⁵² This isolate was selected because it could produce a large number of zoospores needed for column transport experiments. Detailed procedures for producing zoospores is provided in Supporting Information S1. The prepared motile biflagellate zoospore suspensions were hand-shaken vigorously for 90 seconds to induce encystment; shaking was omitted for the motile zoospore suspensions that were used immediately in subsequent column experiments. The zoospore number in the stock suspensions was determined with a hemacytometer and then diluted to obtain the testing zoospore suspensions under desired solution chemistry. The absorbance of the testing suspensions was measured to be 0.174 ± 0.024 at 600 nm by a UV-Visible

Spectrophotometer (Varian Cary 50 Bio, McKinley, New York). The zoospore concentration in the testing suspensions was approximately $4.4 \pm 2.2 \times 10^5$ zoospores mL^{-1} . There was a linear relationship between the zoospore concentration and the absorbance ($r^2 = 0.999$, Figure 2.S1). During dilution, background solution chemistry of the testing zoospore suspensions was adjusted to 0.4 mM NaHCO_3 + 9.6 mM NaCl and 0.4 mM NaHCO_3 + 3.2 mM CaCl_2 ($\text{pH} = 7.2 \pm 0.2$, ionic strength $[\text{IS}] = 10$ mM), respectively. In order to investigate the effect of solution pH on the zoospore transport, the testing zoospore suspensions at $\text{pH} 4.4 \pm 0.1$ were similarly prepared by adjusting solution chemistry to 0.4 mM CH_3COONa + 2.6 mM CH_3COOH + 9.6 mM NaCl and 0.4 mM CH_3COONa + 2.6 mM CH_3COOH + 3.2 mM CaCl_2 ($\text{pH} 4.4 \pm 0.1$, $\text{IS} 10$ mM), respectively. The testing suspensions of motile biflagellate zoospores were prepared at solution pH 7.2 to compare their mobility with that of the encysted zoospores.

ζ -potentials of the zoospore suspensions were measured using a Malvern ZetaSizer Nano ZS (Malvern, Westborough, MA) that employs phase analysis light scattering (PALS) to measure electrophoretic mobility (EPM) of charged particles. The Smoluchowski equation was used to calculate the ζ -potentials from the EPM values. Additionally, the zoospores were fixed by a chemical procedure and imaged by scanning electron microscopy (SEM) as detailed in Supporting Information S1. The SEM images were then analyzed by ImageJ 1.48v (Wayne Rasband, National Institutes of Health, USA) using elliptical and spherical models for the biflagellate and encysted zoospores, respectively. The length of major and minor axes were measured for the biflagellate zoospores, whereas the diameter was measured for the encysted zoospores.

2.2.2. Porous Media

Ottawa sand (99.69% silica, Granusil®) was obtained from Unimin Corporation (Le Seueur, MN). The sand was sieved into fractions of 250–500 μm and 500–804 μm , washed thoroughly as per Bradford et al.,⁵³ dried, and stored in a closed glass bottle. Iron oxide coated sand (IOCS) was prepared following the procedure modified from Schwertmann and Cornell⁵⁴ as described in Supporting Information 2.S1 (Figure 2.S2). Two IOCS with lower and higher iron content (0.11–0.13% and 0.22% by mass) were prepared. The sand grains were mounted on aluminum stubs with carbon cement (SPI supplies, West Chester, PA) to observe the surfaces of uncoated sand and IOCS by SEM. Similar to the previous approach,^{55, 56} the suspensions of IOCS (0.13% Fe) and uncoated sand colloids were generated as described in Supporting Information S1 for the measurements of ζ -potential by the zetasizer.

2.2.3. Column Experiments

Eight column transport experiment sets (Expt. 1.8) were performed to examine the effects of zoospore type (i.e., biflagellate vs encysted zoospores), solution chemistry (i.e., pH and ionic composition), and sand grain properties (i.e., iron oxide coating and grain size) on the mobility of *P. capsici* zoospores in saturated porous media, as shown in Table 2.1. In each experiment, the NaCl and CaCl₂ treatments were conducted in parallel to investigate the effect of cation type in background solution on the zoospore mobility. More specifically, as shown in Table 2.1, Exp. 1 and Exp. 2 compared the difference between the mobility of biflagellate and encysted zoospores. Exp. 3, 4 and 5 and Exp. 2 and 6 examined the effect of iron oxide coating and coated iron content on the transport of encysted zoospores in two sand size fractions (i.e., 250–500 μm and 500–804 μm),

respectively. Exp. 2 and 4 and Exp. 3 and 6 explored the effect of grain size on the transport of encysted zoospores in the presence and absence of iron oxide surface coatings. Finally, Exp. 7 and 8 examined the transport of encysted zoospores at solution pH 4, which was compared with their transport at solution pH 7.2 (i.e., Exp. 3 and 4 in Table 2.1).

A glass column of 12.4 cm in length and 1 cm in inner diameter (Omnifit, Diba Industries, Danbury, CT) was wet-packed with either the uncoated sand or IOCS to a porosity of 0.35. The packed porous media were supported by stainless steel mesh of 104 μm opening size and sealed with O-rings. Two separate columns were used for the zoospore suspensions in 3.2 mM CaCl_2 or 9.6 mM NaCl background solution buffered with 0.4 mM NaHCO_3 (i.e., $\text{pH} = 7.2 \pm 0.2$ and $\text{IS} = 10 \text{ mM}$), which were run at the same time. The zoospore-free background solution was pumped through the column using a syringe pump (Model 351, Sage Instruments, White Plains, NY) at about 0.2 mL min^{-1} for 45 minutes to equilibrate the column. The testing zoospore suspension of $3.1 \pm 2.0 \times 10^5$ zoospores mL^{-1} or a solution of bromide tracer at 50 mg L^{-1} was injected for 30 minutes at the identical flow rate, followed by injecting the background solution for 90 minutes. An infusion and withdrawal syringe pump (Model 74905-54, Cole-Parmer, IL) was used in the bromide tracer experiment. The average pore water velocity was $0.77 \pm 0.04 \text{ cm min}^{-1}$. The effluent samples were collected in 5-minute intervals using a fraction collector (Retriever 500, Teledyne ISCO) and measured for absorbance at 600 nm for the zoospore effluent samples and at 204 nm for the bromide effluent samples using the UV-visible spectrophotometer. The background absorbance (**0.0004 ± 0.0005**) of the effluents was subtracted from the effluent sample absorbance. The breakthrough curves were

plotted as normalized effluent concentrations as a function of pore volumes. Effluent mass recovery (M_{ER}) was calculated by integrating the BTCs using the trapezoidal rule and then dividing the recovered mass by the input mass. The transport of zoospores or the conservative Br tracer is described by a convection-dispersion equation with a first-order kinetic deposition term.

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial z^2} - v \frac{\partial C}{\partial z} - k_d C \quad (1)$$

where C is the liquid phase concentrations, t is the elapsed time, D is the hydrodynamic dispersion coefficient, z is the travel distance, v is the pore water velocity, and k_d is the deposition rate coefficient. For the bromide tracer k_d is equal to zero. Thus, the bromide BTC was fitted to the convection-dispersion equation to characterize hydrodynamic properties of the column (Figure 2.S3). The estimated D value was $0.055 \text{ cm}^2 \text{ min}^{-1}$, and the flow regime in the columns was convection-dominant, characterized by a high Péclet number ($Pe = vL/D = 161$, where L is the column length).^{57, 58} Therefore, the dispersion term in Eq. 1 can be ignored, and k_d was estimated as follows:^{57, 59, 60}

$$k_d = -\frac{v}{L} \ln(M_{ER}) \quad (2)$$

At the end of column experiments, the zoospores attached on IOCS were taken out from the column using spatula, and observed with SEM. Briefly, the samples were fixed at 4°C for 12 hours in a 4% glutaraldehyde solution buffered with 0.1M sodium phosphate at pH 7.4. Following a brief rinse in the buffer, samples were dehydrated in an ethanol series (25%, 40%, 50%, 60%, 75%, and 95%) for 30 minutes at each gradation and with three 30-min changes in 100% ethanol. The remaining procedure was the same as that for the zoospore SEM imaging.

Table 2.1. Characteristics of *P. capsici* Zoospore Suspensions and Their Transport Parameters through Saturated Columns Packed with Iron Oxide Coated Sand (IOCS) and Uncoated Sand.^a

Experiment (n)	Zoospore type	Media Type	Solution pH	Cation Type	EPM ($\mu\text{m cm V}^{-1}\text{s}^{-1}$)	ζ -potential (mV)	M_{ER}	k_d (min^{-1})
Exp. 1 (n =2)	Biflagellate zoospores	IOCS, 500–804 μm , 0.11% Fe	7.2 ± 0.2	Na^+	-1.41 ± 0.11 cd	-18.0 ± 1.4 cd	0.139 ± 0.015 a	0.122 ± 0.011 f
				Ca^{2+}	-0.92 ± 0.06 ab	-11.8 ± 0.7 ab	0.018 ± 0.013 de	0.260 ± 0.043 abc
Exp. 2 (n =3)				Na^+	-1.30 ± 0.17 c	-16.6 ± 2.2 c	0.030 ± 0.009 cd	0.216 ± 0.023 cde
				Ca^{2+}	-0.75 ± 0.05 a	-9.5 ± 0.7 a	0.014 ± 0.002 de	0.266 ± 0.016 ab
Exp. 3 (n =3)	Encysted zoospores	Sand, 250~500 μm , 0% Fe		Na^+	-1.43 ± 0.08 cd	-18.2 ± 0.8 cd	0.059 ± 0.008 b	0.174 ± 0.004 ef
				Ca^{2+}	-1.09 ± 0.20 b	-13.9 ± 2.5 b	0.058 ± 0.016 b	0.168 ± 0.017 ef
Exp. 4 (n =3)		IOCS,250~500 μm , 0.13% Fe		Na^+	-1.55 ± 0.11 d	-19.8 ± 1.4 d	0.026 ± 0.012 cde	0.227 ± 0.036 bcd
				Ca^{2+}	-1.06 ± 0.06 b	-13.5 ± 0.8 b	0.023 ± 0.008 cde	0.241 ± 0.035 bcd
Exp. 5 (n =2)		IOCS, 250~500 μm , 0.22% Fe		Na^+	-1.43 ± 0.04 cd	-18.3 ± 0.5 cd	0.011 ± 0.003 de	0.312 ± 0.011 a
				Ca^{2+}	-1.05 ± 0.25 b	-13.4 ± 3.3 b	0.007 ± 0.006 e	0.310 ± 0.046 a
Exp. 6 (n =3)		Sand, 500~804 μm , 0% Fe		Na^+	-1.33 ± 0.06 c	-16.9 ± 0.8 c	0.042 ± 0.017 bc	0.208 ± 0.038 cde
				Ca^{2+}	-0.81 ± 0.09 a	-10.3 ± 1.1 a	0.042 ± 0.016 bc	0.205 ± 0.027 de
Exp. 7 (n =1)		Sand, 250~500 μm , 0% Fe	4.4 ± 0.1	Na^+	-0.77	-9.9	0.019	0.243
				Ca^{2+}	-0.61	-7.9	0.007	0.314
Exp. 8 (n =1)		IOCS, 250~500 μm , 0.13% Fe		Na^+	-0.78	-9.9	0.004	0.334
				Ca^{2+}	-0.60	-7.7	0.002	0.383

^a n = number of replicates, EPM = electrophoretic mobility, M_{ER} = effluent mass recovery, k_d = deposition rate coefficient, and means in a column with different lower case letters are significantly different ($P < 0.05$) using the Least Significant Difference (LSD) method.

2.2.4. XDLVO Calculations

Surface energies of zoospores interacting with uncoated sand or IOCS surfaces were calculated according to the extended Derjaguin-Landau-Verwey-Overbeek (XDLVO) theory, including Lifshitz-van der Waals, electrical double layer, and Born repulsion interactions. Detailed calculations are provided in the Supporting Information S2. The XDLVO interaction energies determine zoospore attachment strength on grain surfaces at the primary and second energy minima, quantified by attachment efficiencies (α). Then, theoretical k_d values and effluent recoveries were estimated to compare with the experimental results, thus allowing the assessment of the contribution of zoospore attachment on the grain surface (i.e., the solid-water interface) to overall zoospore retention.

2.3. RESULTS AND DISCUSSION

2.3.1. Characterization of Porous Media and Zoospores

The prepared IOCS had a brownish color (Figure 2.S2) indicative of iron oxide coating. A closer examination of the IOCS surface by SEM revealed increased surface roughness compared with the uncoated sand due to nano-sized features of iron oxide (Figure 2.S4), as confirmed by energy-dispersive X-ray spectroscopy (Figure 2.S5). The determined elemental Fe content was 0.003% for the uncoated sand, 0.11–0.13% for the IOCS of lower Fe content, and 0.23% for the IOCS of higher Fe content, respectively. At a near neutral pH of 8.0–8.5, the surface of IOCS became less negatively charged with increasing Fe content, and in CaCl_2 solution, surface charge was even reversed to the positive sign (Table 2.S1). The observed charge reversal likely resulted from either Ca^{2+}

adsorption on or accumulation near the IOCS surface.⁶¹⁻⁶³ At the lower pH of 3.8, surface charges of zoospore and porous media were much less negative, and the IOCS surface became positively charged, due to protonation of IOCS surface. As the isoelectric point of quartz and iron oxide was typically 1.5–3.0 and 7.5–9.0, respectively,⁶⁴ iron oxide surface was less negatively charged than quartz sand at identical solution pH and IS (Table 2.S1). On average the IOCS surface had much less electrostatic repulsion and probably even attraction to the negatively charged zoospores (Table 2.1). Consequently, iron oxide coating often serves as favorable retention sites for negatively charged particles such as *Escherichia coli* and *Cryptosporidium parvum* oocysts.⁶⁵⁻⁶⁷

Representative SEM images of biflagellate and encysted *P. capsici* zoospores in CaCl₂ solutions at pH 7.2 and IS 10 mM are shown in Figure 2.1. More SEM images of the zoospores in DI water and NaCl solutions are provided in Figure 2.S6 and 2.S7. Biflagellate zoospores were of ovoid shape (Figure 2.1, 2.S6, and 2.S7) with major axis of $8.2 \pm 1.0 \mu\text{m}$, minor axis of $5.7 \pm 0.5 \mu\text{m}$, aspect ratio of 1.4 ± 0.2 , and roundness of 0.7 ± 0.1 , as measured by ImageJ. During encystment, the zoospores lost two flagella, and were transformed into nearly a spherical shape with diameter of $6.9 \pm 0.4 \mu\text{m}$, aspect ratio of 1.0 ± 0 , and roundness of 1.0 ± 0 , similar to other *Phytophthora* zoospores.^{46, 47} Figure 2.S6C and Figure 2.S7B showed the shape of the zoospore in transition after losing two flagella and prior to formation of the spherical encysts. Encysted zoospores in the testing suspensions primarily consisted of the spherical shape; the presence of the ovoid-shaped transitory encysts were minor, and the biflagellate zoospores were almost nonexistent (Figure 2.1 and 2.S6). The surface of encysted and biflagellate zoospores appeared rough and covered with extracellular surface coats probably containing acidic

surface groups^{68, 69} and polysaccharides.⁷⁰ Another important change during encystment was reported to be the secretion of adhesive materials such as glycoprotein from peripheral vesicles and the formation of the outer surface coating of the cysts.⁷¹⁻⁷³

As expected, under identical solution pH and IS, both biflagellate and encysted *P. capsici* zoospores were less negatively charged in CaCl₂ solution than in NaCl solution (Table 2.1), which likely resulted from Ca²⁺ adsorption on or accumulation near the zoospore surface. Surface charges of colloids and collector grains determine the electrical double layer force important to colloid retention in porous media.²³

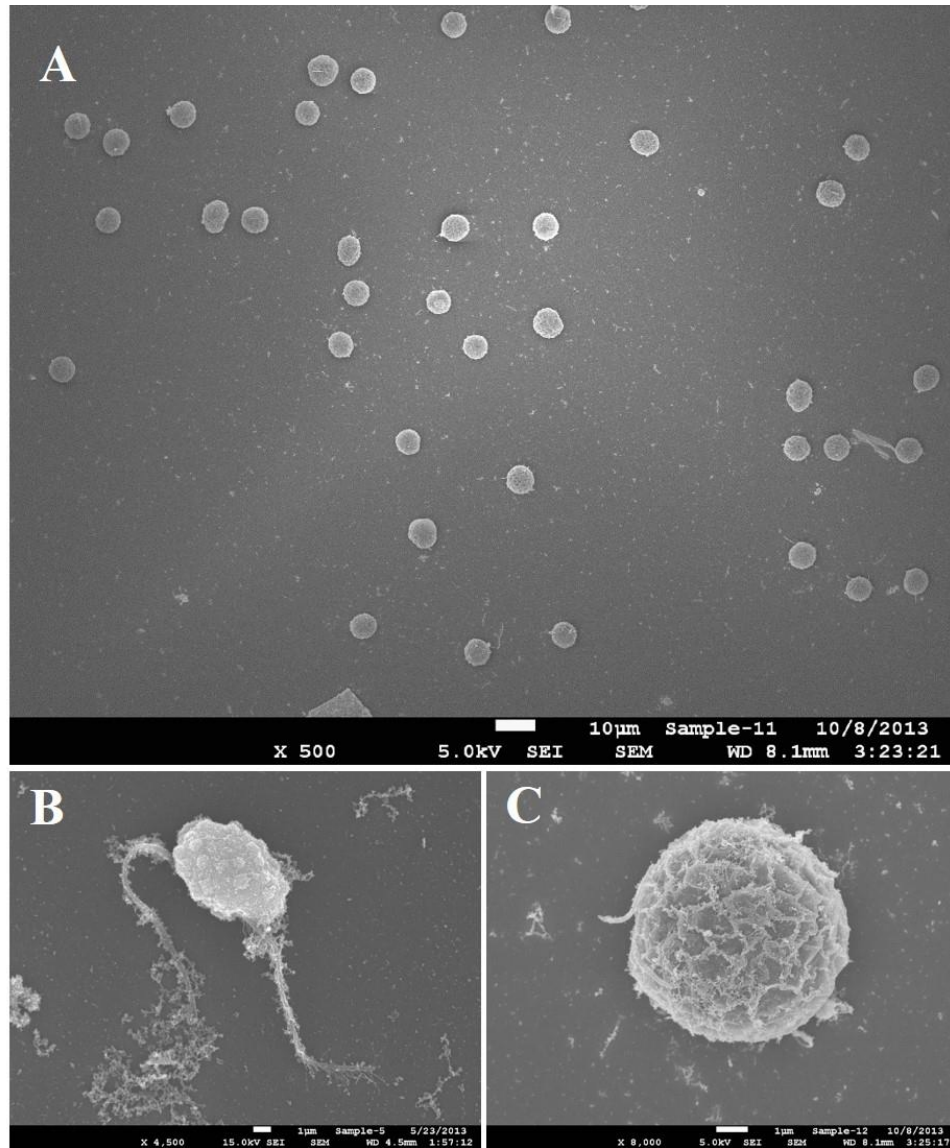


Figure 2.1. Representative SEM images of encysted (A and C) and biflagellate (B) *P. capsici* zoospores in CaCl_2 solutions at pH 7.2 and IS 10 mM.

2.3.2. Retention of Encysted Zoospores

Biflagellate zoospores encyst within a few hours even in a quiescent suspension and it was expected that the zoospores in water flows over longer spatial and time scales would be the cysts most of time. Thus, the majority of the experiments focused on the encysted zoospores. The influences of physicochemical properties of porous media and solution chemistry on the transport of zoospores are discussed along with the retention mechanisms for *P. capsici* zoospores.

2.3.2.1. Effect of Iron Oxide Coating and Grain Size

The presence of iron oxide surface coating significantly enhanced the retention of encysted zoospores under identical solution chemistry as shown Figure 2.2. Examination of M_{ER} and k_d values for Expt. 2 and 6 revealed that in the 500-804 μm fraction the zoospore transport through uncoated sand was significantly greater than through IOCS in Ca^{2+} background solution, but no significant differences were observed in Na^+ background solution (Table 2.1). In the 250–500 μm fraction for Expt. 3, 4, and 5, the zoospores were significantly less retained in the uncoated sand than in IOCS in both Na^+ and Ca^{2+} background solutions (Table 2.1). It was noted that Figure 2.2A showed a greater zoospore retention in the IOCS of 0.22% Fe than the IOCS of 0.13% Fe supported by post hoc multiple comparisons of k_d values (Table 2.1), but the M_{ER} statistics did not show the same. Because the M_{ER} data were not normally distributed, the post hoc tests for M_{ER} by the Least Significant Difference (LSD) method might not be valid in some cases and thus not discussed hereafter. My results showed that iron oxide surface generally facilitates the retention of biocolloids, agreeing with previous studies for *E. coli*⁶⁷ and *Cryptosporidium parvum* oocysts.^{65, 66} This observation was also in line with the ζ -

potential measurements as discussed previously (Table 2.S1) and the XDLVO calculations showing less repulsive energy profiles for the zoospore interacting with IOCS (Table 2.S2 and Figure 2.S9). Hence, for soils with greater iron oxide contents such as Spodosol, Oxisol and Ultisol, the transport of zoospores would be much more limited. Similarly, engineered filter media coated with nano-sized iron oxide could also be employed to increase the retention of zoospores.

Interestingly, there was no significant difference of zoospore retention between the 250–500 μm and 500–804 μm fractions (i.e., Expt. 2 vs Expt. 4 and Expt. 3 vs Expt. 6, Table 2.1). The ratio of zoospore to collector diameter was 0.009–0.0276, which is much greater than the threshold ratio of 0.003 under which significant straining would occur.⁷⁴⁻⁷⁷ Therefore, straining played an important role in zoospore retention. However, XDLVO calculations also suggested strong surface attachment of zoospores to uncoated sand and IOCS with attachment efficiencies (α) ranging 0.996–1.000. Thus, the coupling between chemical and physical factors is expected to influence distribution of retained zoospores on grain surface and pore straining sites.⁷⁸⁻⁸¹ Given the strong zoospore retention from surface attachment, decreasing grain size did not show a significant effect on zoospore retention.

2.3.2.2. Effect of Solution Chemistry

There was no significant difference in the transport of encysted zoospores through uncoated sand and IOCS of 250–500 μm in either NaCl or CaCl₂ background solution (Table 2.1 and Figure 2.2). These observations were contrary to the decreased ζ -potentials of zoospores (Table 2.1) and grain surface (Table 2.S1). XDLVO calculations suggested almost complete retention of encysted zoospores, due to either the deep

secondary minima or the absence of primary maxima (Figure 2.S9 and Table 2.S2). Indeed, the estimated effluent recovery was 0–0.006 and much lower than most experimental M_{ER} values in Table 2.1. Therefore, the XDLVO calculations could not fully explain the zoospore transport. The deficiencies of XDLVO theory could be explained by its limitations for biological colloids due to more complex surface properties, and by its inability to account for colloid retention by pore straining. Intriguingly, the k_d value of the zoospores in the IOCS of 500-804 μm was significantly higher in Ca^{2+} background solution than Na^+ background solution. In this case, stronger XDLVO attractive force and less colloid straining due to larger collector grain size are expected. Because colloid retention is highly dependent on the coupled effect of surface attachment and pore straining,^{78, 79, 82} it is likely that the increased contribution from surface attachment in the 500-804 μm IOCS made it possible to observe the greater retention in Ca^{2+} background solution. Indeed, Bradford et al.⁷⁵ reported that the contribution of straining to the retention of similarly-sized *Cryptosporidium* oocysts (5 μm) was decreased from 79% for 360- μm sand to 68% for 710- μm sand. Lowering solution pH increased the retention of encysted zoospores (Figure 2.3) due to protonation of both sand and zoospore surfaces and subsequently increased electrostatic attraction, as observed for other microorganisms.^{65, 83} XDLVO calculations showed a mostly favorable condition for zoospore deposition (Table 2.S2, Figure 2.S9), indicating that XDLVO calculation agreed with the experimental results reasonably well, despite its inherent limitations.

However, it should be noted that despite almost complete retention predicted by XDLVO calculations and strong straining, a small percentage of encysted zoospores (<

5.9%, Table 2.1) still passed through the column. Therefore, the XDLVO theory and straining could not fully explain the zoospore transport and retention behaviors. This was likely because rough outer surfaces (including glycoproteins and polysaccharides) of the zoospores provided steric repulsion that is not included in XDLVO calculations, and this steric repulsion could reduce the zoospore retention.

Encysted zoospores were also found to be attached on the IOCS surface through filamentous materials (Figure 2.4 and 2.S8). Encysting zoospores released adhesive content, likely glycoproteins, from peripheral vesicles to coat the cell surface and anchor the zoospores to a solid surface.⁷¹⁻⁷³ While it was argued that this type of attachment mechanism occurred in the early stage of encystment,⁷² this study showed that secreted adhesive materials on the cell surface could still attach the zoospores to the solid surface long after encystment, thus partly contributing to the zoospore retention in the porous media. Overall, the retention of zoospores in the porous media was collectively controlled by electrostatic, steric and adhesive surface interactions, and pore straining.

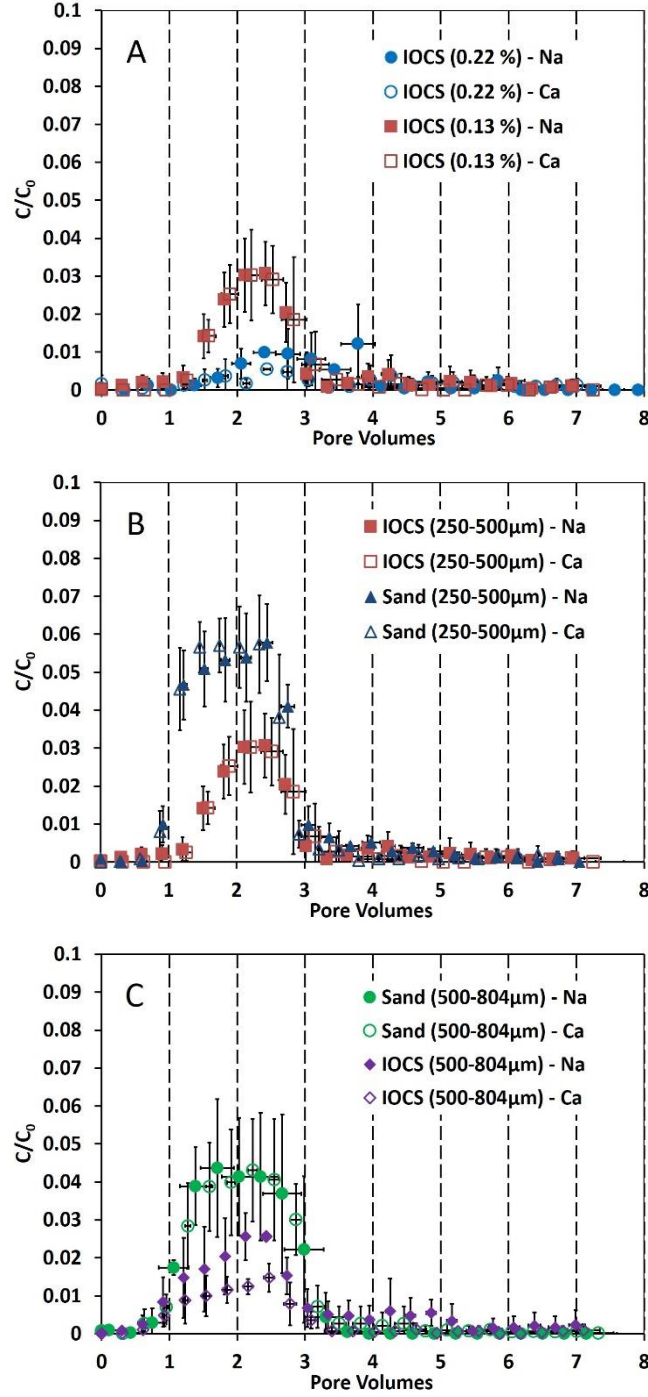


Figure 2.2. Breakthrough curves of encysted *P. capsici* zoospores in NaCl or CaCl₂ solutions (pH 7.2 and IS 10 mM) through saturated columns packed with IOCS (250–500 μm) of varying iron oxide coatings (A), IOCS and uncoated sand of 250–500 μm (B) and 500–804 μm (C).

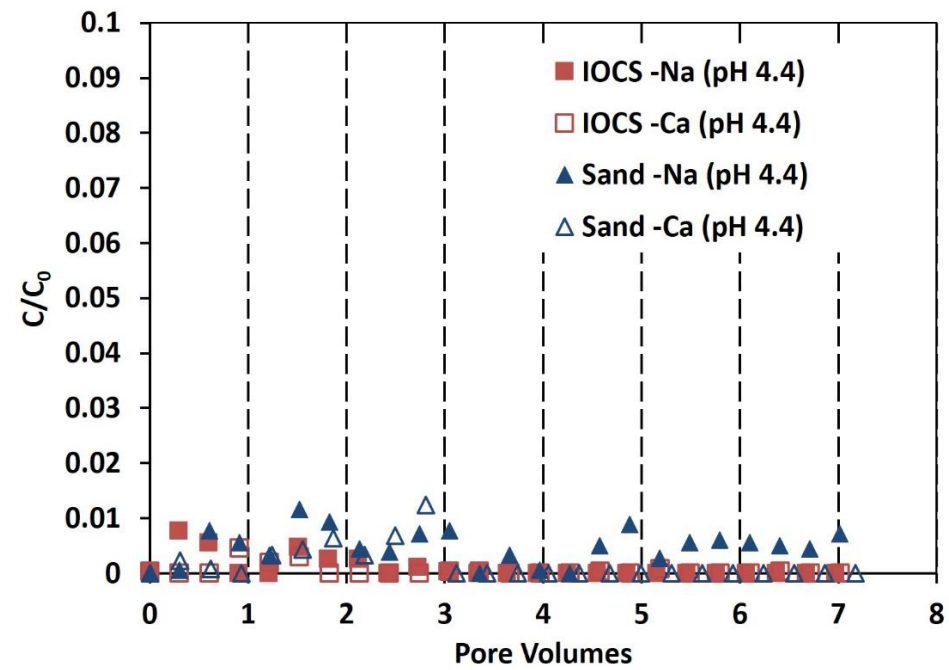


Figure 2.3. Breakthrough curves of encysted *P. capsici* zoospores through saturated columns packed with IOCS and uncoated sand at solution pH 4.4.

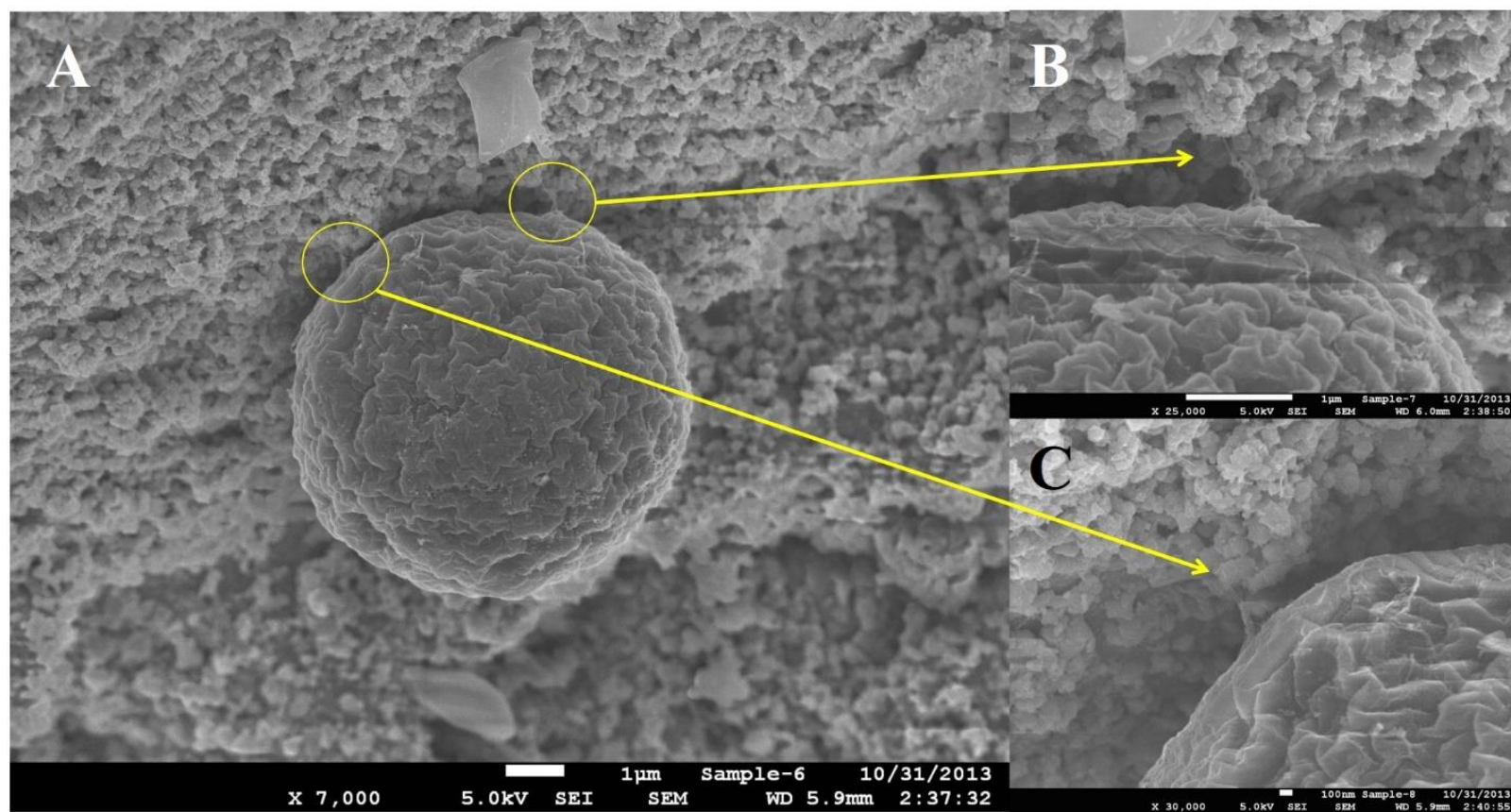


Figure 2.4. SEM images of encysted *P. capsici* zoospore attached on the IOCS surface in the presence of Ca^{2+} .

2.3.2.3. Comparison of Biflagellate and Encysted Zoospores

Because both biflagellate and encysted zoospores of *P. capsici* could be present in contaminated water, I examined their transport behaviors under otherwise identical experimental conditions. In the coarse IOCS, the retention of biflagellate zoospores was much less than that of encysted zoospores in NaCl solution (Figure 2.5), and the k_d value was 0.122 ± 0.011 (Exp. 1, Table 2.1), relative to the k_d value of 0.216 ± 0.023 for the encysted zoospores (Exp. 2, Table 2.1). Thus, when the biflagellate zoospores were initially released from the sporangia, they were suited for enhanced mobility in porous media. This observation agrees with the study of Wilkinson et al.⁴⁰, reporting that *Phytophthora megasperma* motile zoospores were transported much further than its nonmotile cysts in unsaturated sand, sandy clay loam, and loam soils. It could be argued that this mobility difference likely resulted from avoidance of the retention sites (e.g., low flow regions or pore straining sites) by motile zoospores.^{22, 40} It could also be due to the lack of secreted adhesive materials such as glycoprotein on the outer surface of the biflagellate zoospores,²² as the adhesion of zoospores on the IOCS surface by the adhesives (Figure 2.4 and 2.S8) was identified as one of retention mechanisms. In the presence of Ca^{2+} , the mobility of biflagellate zoospores was significantly decreased and indistinguishable from that of encysted zoospores (Table 2.1 and Figure 2.1). This could result from the inhibitory effect of Ca^{2+} on the zoospore motility due to increased encystment in the presence of Ca^{2+} ,⁸⁴ which would result in lower motility and increased adhesive surface coats. It is possible that these mechanisms collectively contributed to the reduced retention of biflagellate zoospores.

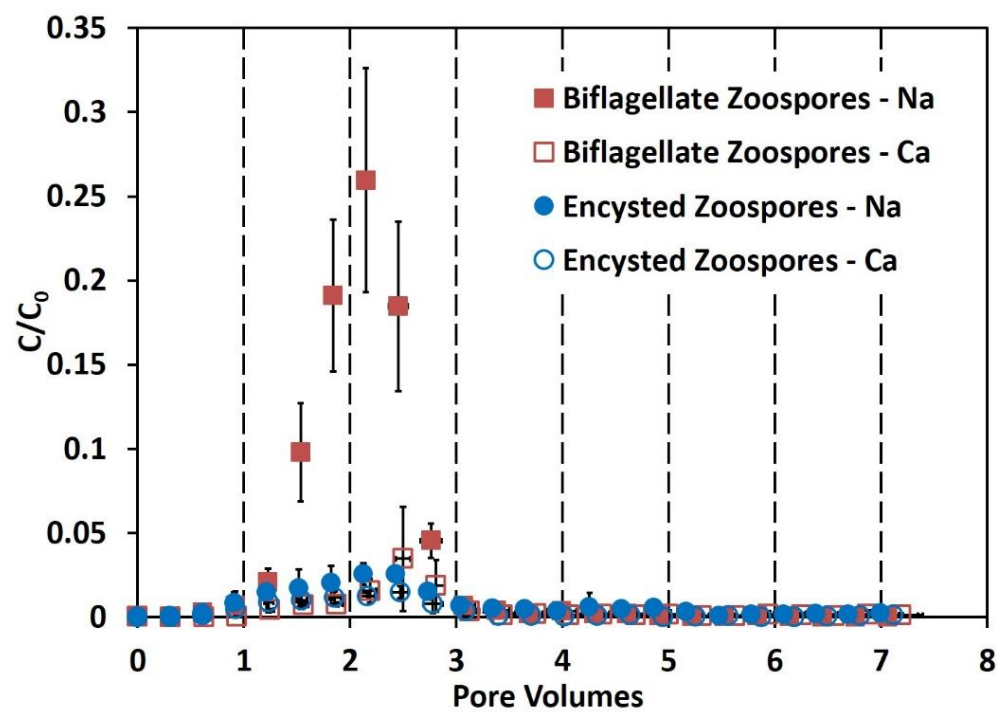


Figure 2.5. Breakthrough curves of biflagellate and encysted *P. capsici* zoospores through saturated columns packed with IOCS (500–804 μm).

2.4. ENVIRONMENTAL IMPLICATIONS

My findings suggest that the transport of the plant pathogen *P. capsici* zoospores in porous media was collectively controlled by surface properties of zoospores and porous media, solution chemistry, and pore straining. Because zoospore retention in iron-oxide coated sand or at lower solution pH was significantly enhanced, limited zoospore dispersal through iron-oxide rich (e.g., Spodosol, Ultisol, and Oxisol) or acidic soils is expected. For encysted zoospores, their mobility in soils is low because of their low transport in the coarse sand tested in this study. However, considering the high transport potential of motile biflagellate zoospores even in iron-oxide coated sand, the motile phase of the zoospores appeared to be a period of substantial dispersal and infection risk, likely during the first several hours of rainfall or irrigation events. Therefore, strategies to control the water-induced *Phytophthora* infection from the zoospores could focus on the short period following the water events. Given that the mobility difference of motile and nonmotile zoospores was not observed in Ca^{2+} background solution, one effective strategy to reduce pathogen transport is to induce zoospore encystment by increasing Ca concentrations in soil water via liming. Additionally, this study also indicate that it is possible to design filter media effective in removing plant pathogens from irrigation water, which could be highly beneficial to the recirculating greenhouse production systems. For the easy and precise detection by optical density, I used high zoospore input concentrations of $4.4 \pm 2.2 \times 10^5$ zoospores mL^{-1} , and filtration reduced the peak zoospore effluent concentration to about 2,000–17,000 zoospores mL^{-1} (i.e., about 1.2–2.1 Log reduction). Because water containing ≥ 5000 zoospores/mL could infect 100% of pickling cucumbers (*Cucumis sativus*) at temperatures above 12 °C,⁸⁵ the small

percentage of transported zoospores might still pose a substantial risk to susceptible crops. Hence, future research should be directed to investigate the transport behaviors of zoospores at low input concentrations. Also, this study investigated one type of plant pathogen in model porous media, and future studies should be conducted to examine other types of plant pathogens in more realistic conditions.

2.5. ACKNOWLEDGMENTS

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APPENDIX

APPENDIX

SUPPORTING INFORMATION

S1. Preparation and Characterization of *P. capsici* Zoospores and Porous Media

S1.1. Preparation of Zoospore Suspension

To produce *P. capsici* zoospores, the culture was grown on V-8 agar (UCV8), which consisted of 840 mL distilled water, 160 mL unclarified V8 juice, 30 mM CaCO₃, and 1.5% agar. The isolate was maintained on UCV8 at 25°C under continuous fluorescent lighting. After 7–8 days, sterile distilled water was added to the culture, *P. capsici* sporangia were scraped from the surface of the agar, and the resulting suspension was placed into a 50- mL centrifuge tube. The tube was incubated at 4°C for 30–45 minutes and then at room temperature for 30 minutes to allow for zoospore release from sporangia. Since zoospores are negatively geotropic, a stock zoospore suspension was obtained by taking the top portion of the suspension, which excluded solid growth media, hyphal fragments, and sporangia. To determine the concentration of the zoospore suspension, a 1-mL aliquot was placed into a 1.7-mL microcentrifuge tube, vortexed for 70 seconds to induce zoospore encystment, and a 10-μL aliquot was pipetted onto a clean hemacytometer for counting (Bright-Line, Hausser Scientific, Horsham PA).

S1.2. SEM Imaging of Zoospores

To observe the zoospores by scanning electron microscope (SEM), 8 drops of the zoospore suspension was mixed with an equal quantity of 4% glutaraldehyde buffered at pH 7.4 with 0.1 M sodium phosphate. Fixation was allowed to proceed for 1 hour at 4°C. A 12-mm round glass coverslip coverslip was coated with Poly-L-Lysine (Sigma Aldrich P1399) by pressing a drop of 1% Poly-L-Lysine solution downward on a plastic petri dish

for 10 minutes and then gentle rinsing with several drops of water. One drop of the zoospores fixed in suspension was placed on the wet Poly-L-Lysine coated coverslip surface and settled for 10 minutes. The coverslip was then gently rinsed with several drops of water, sequentially immersed in 25%, 40%, 50%, 60%, 75%, and 95% ethanol solution for 10 minutes, and finally placed in 100% ethanol for 10 minutes for three times. The samples were dried in a critical point dryer (Balzers Model 010, Balzers Union Ltd., Balzers, Liechtenstein) using liquid CO₂ as the transitional fluid, mounted on aluminum stubs with carbon suspension cement (SPI Supplies, West Chester, PA), coated with osmium (≈15 nm thickness) in a NEOC-AT osmium coater (Meiwafosis Co., Ltd., Osaka, Japan), and finally examined by a SEM with cold field emission electron emitter (JEOL JSM-7500F, JEOL Ltd, Tokyo, Japan).

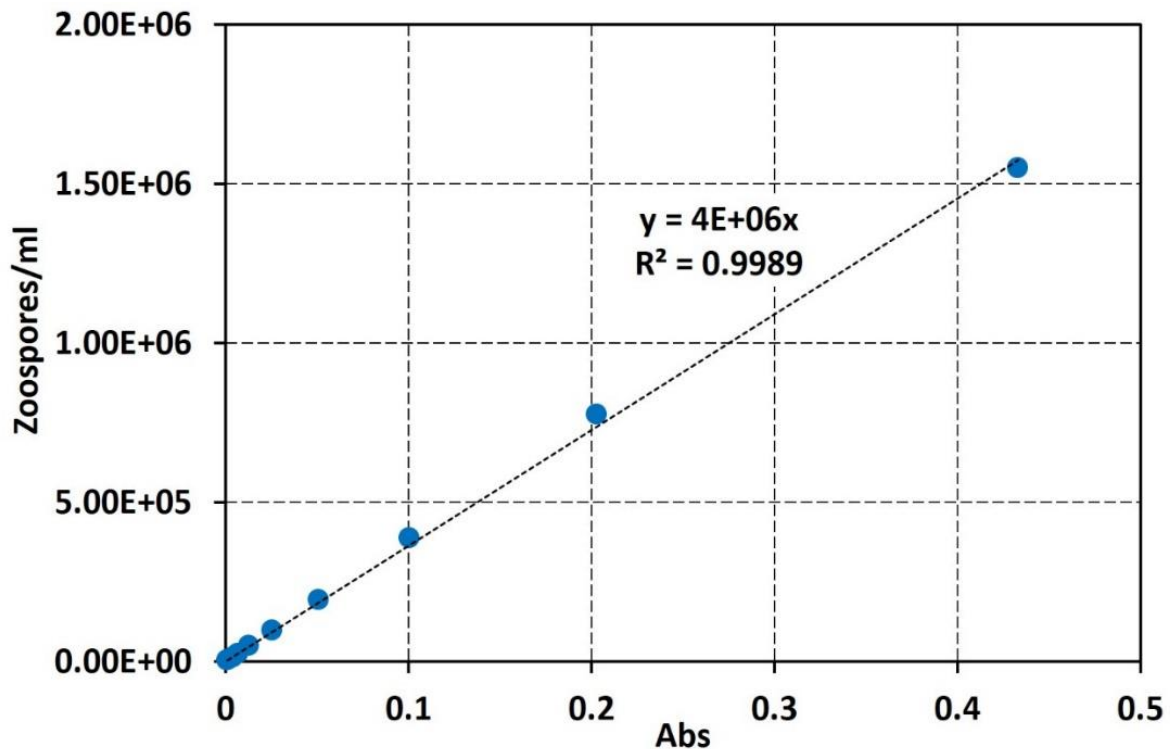


Figure 2.S1. Observed *P. capsici* zoospore number concentrations in relation to absorbance.

S1.3. Iron Oxide Coating of Sand

To produce iron oxide coated sand, 200 gram of clean sand was added to a mixture of 200-mL 0.05 M ferrous chloride solution and 40-mL 1 M NaHCO₃ solution. The slurry was stirred for 1.5 hr with magnetic bar to introduce oxygen into the suspension and achieve uniform coating. During this process, Fe (II) in the slurry was gradually oxidized to Fe (III), as shown by the color changes in Figure 2.S2B. After 1.5 hours of mixing, the coated sand was washed using copious amount of DI water, and then oven dried at 110 °C for 24 hours. Finally, a reddish-brown coating was formed (Figure 2.S2C). This procedure was repeated four times in total in order to increase Fe content of the coated sands. To create IOCS with a greater iron content, the mixture was stirred at a higher speed with a stirrer (RW11, IKA, Germany) for a longer time. To determine iron content, IOCS was soaked in 2.0 M HCl for 48 hours at room temperature until coated iron was completely dissolved. Iron concentration in the prepared solutions was measure by atomic absorption spectroscopy (AAnalyst 400, PerkinElmer, Waltham, MA). To measure the ζ -potentials of IOCS and uncoated sand surfaces, a 5-gram sand sample was ultrasonicated in 15 mL deionized (DI) water for 30 minutes and then vortexed to re-suspend sand particles prior to the 206-min settling. The top 5-cm suspension was taken for the ζ -potential measurements. This suspension approximately contained sand colloids smaller than 2 μ m, assuming the Stokes' law and particle density of 2.65 g/cm³. Each ζ -potential measurement was repeated six times and the average value was calculated.

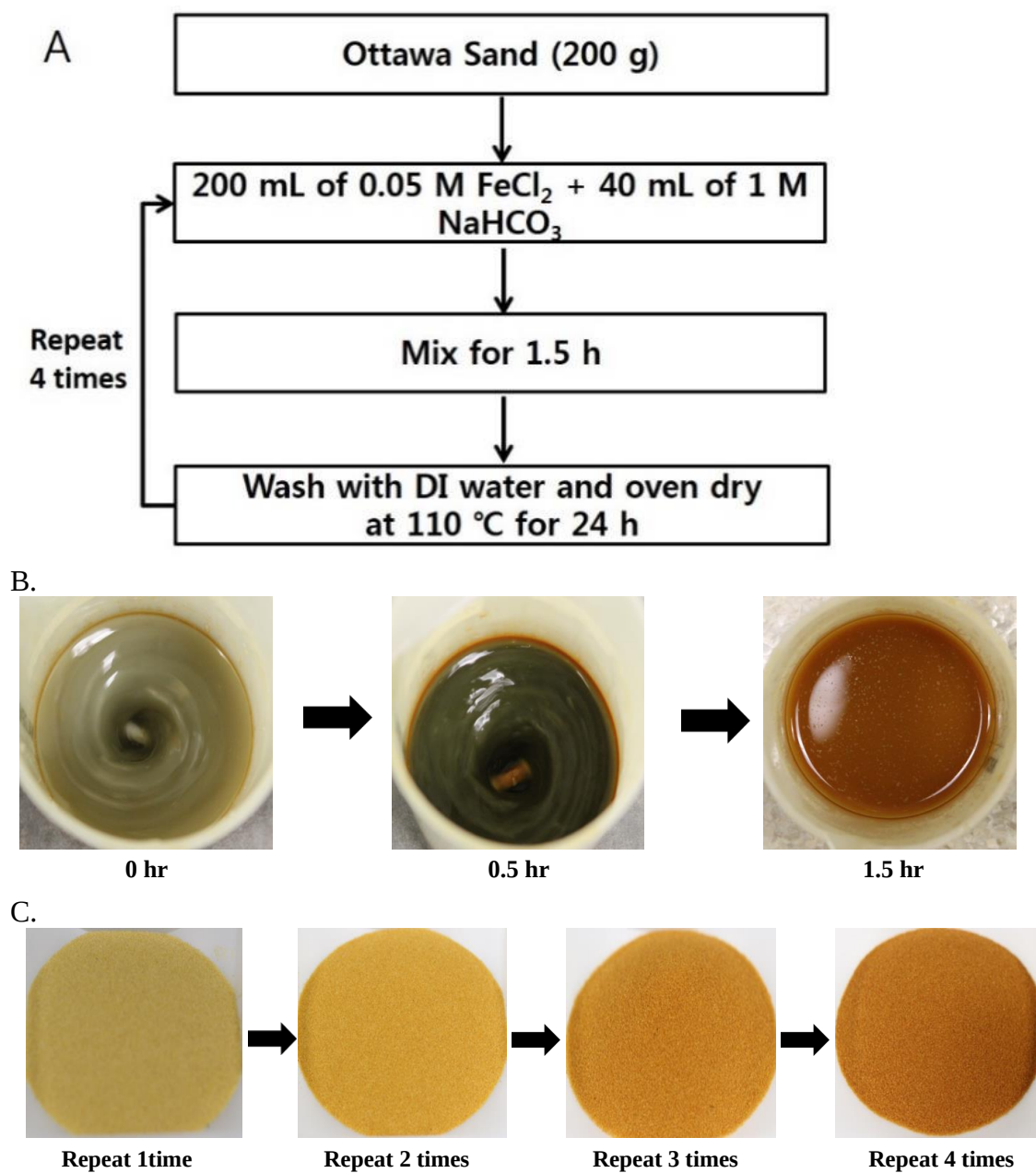


Figure 2.S2. Schematic of preparation procedure for iron oxide coated sand (IOCS) (A), and temporal visual changes of the preparation slurry (B) and IOCS (C).

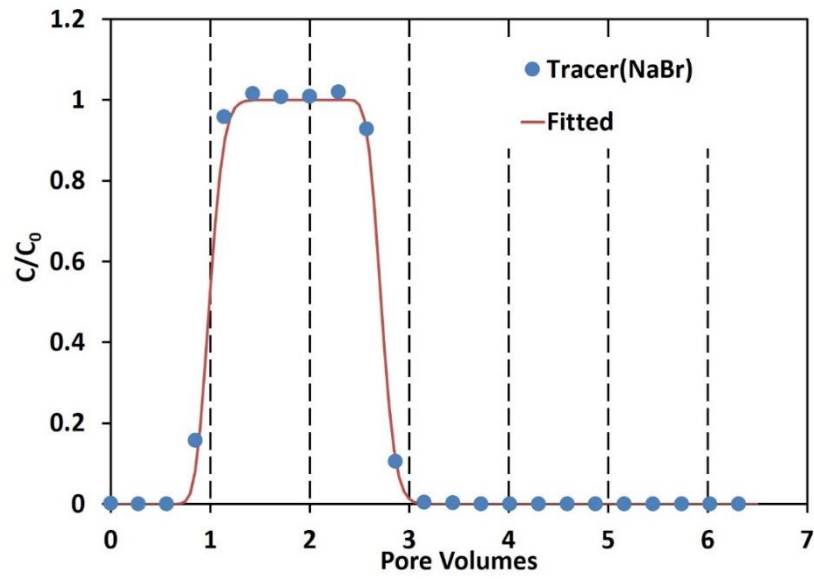


Figure 2.S3. Observed and fitted breakthrough curves of bromide tracer through saturated column packed with uncoated sand (250–500 μm).

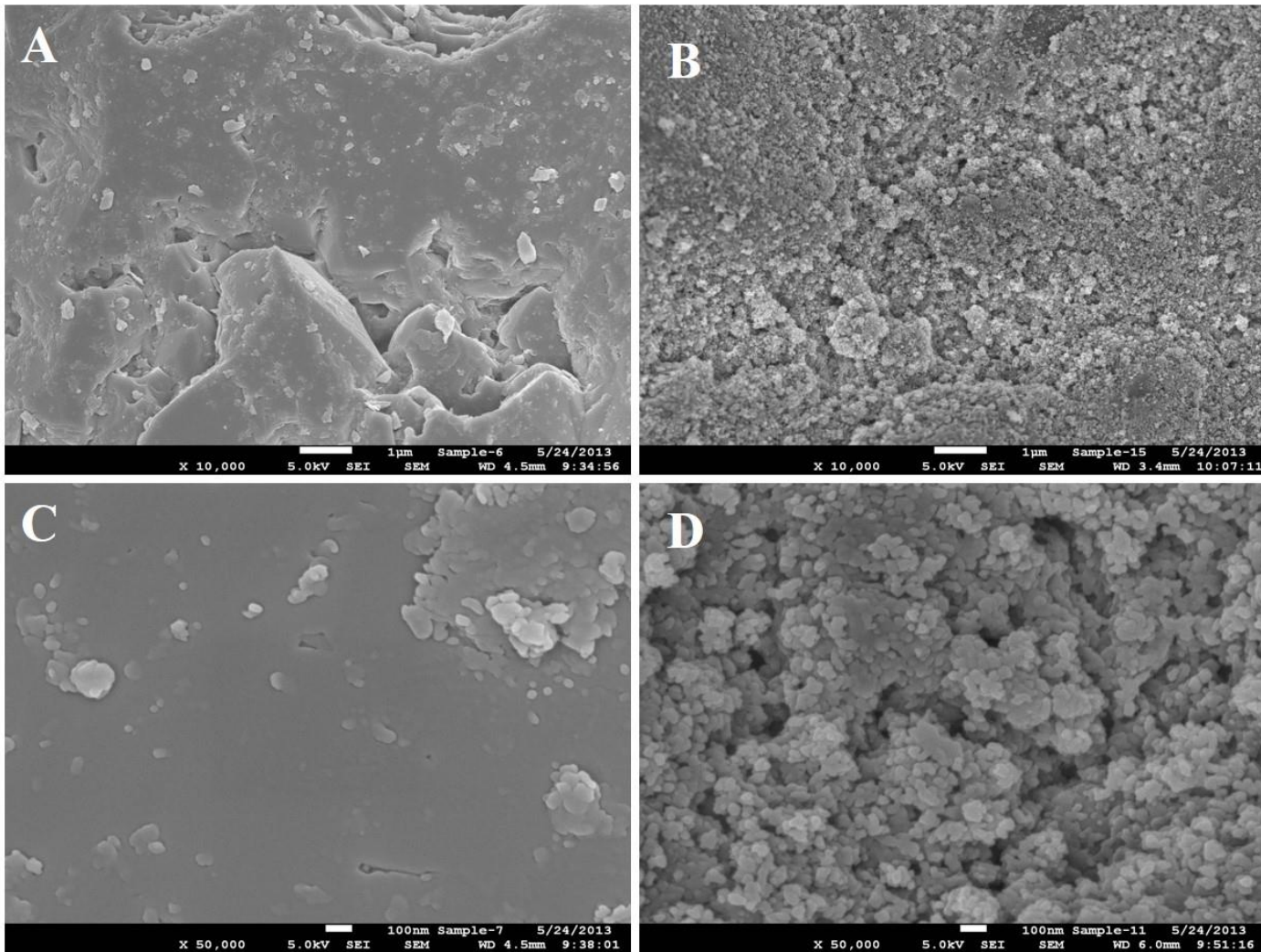


Figure 2.S4. SEM images of Ottawa sand (A: $\times 10,000$ and C: $\times 50,000$) and iron oxide coated sand (B: $\times 10,000$ and D: $\times 50,000$) of 250–500 μ m.

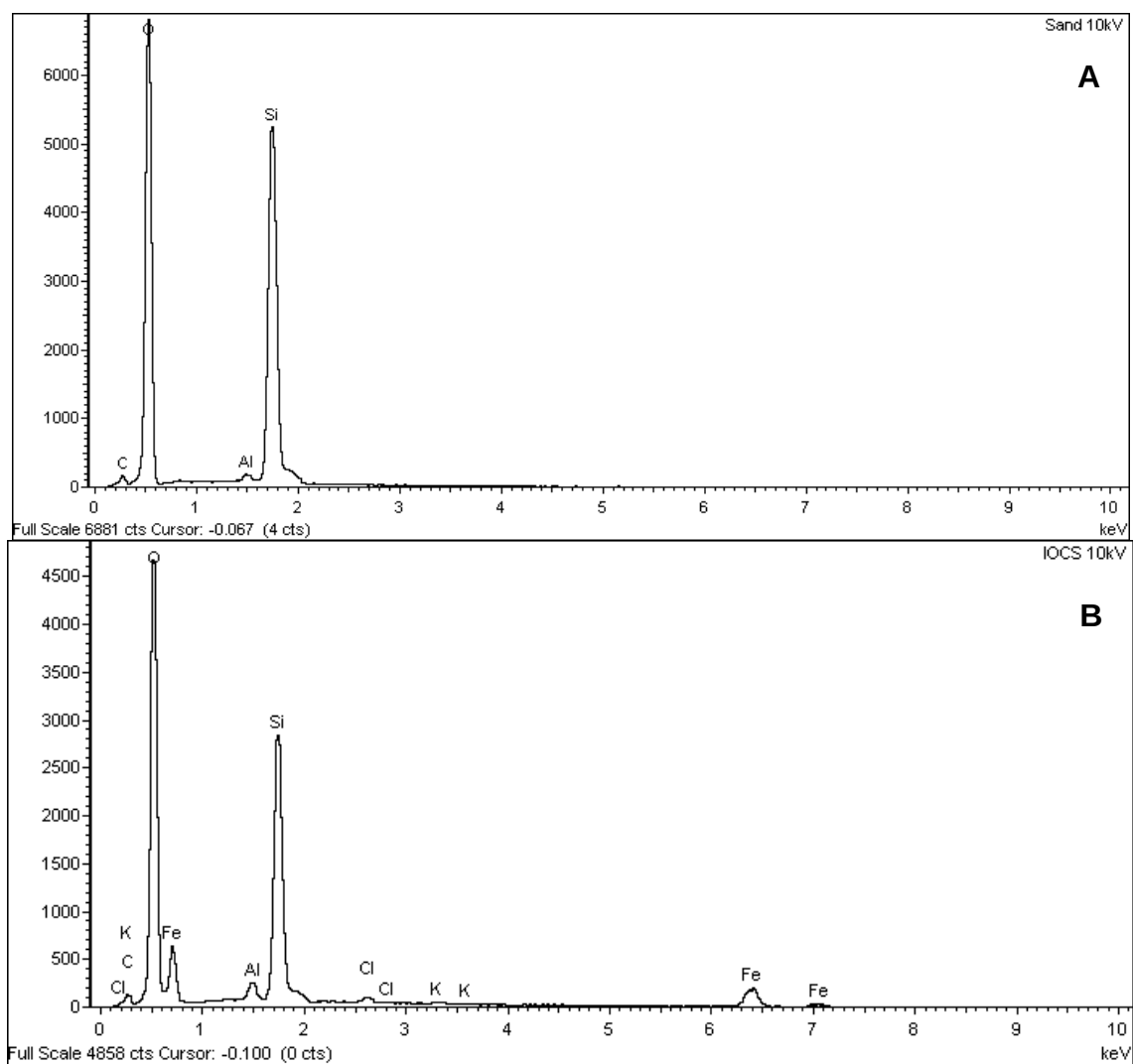


Figure 2.S5. Energy-dispersive X-ray spectra of uncoated (A) and iron oxide coated sand (B).

Table 2.S1. Electrophoretic Mobility (EPM) and ζ -potential of Uncoated and Iron Oxide Coated Sand.

Solution chemistry	pH	Uncoated sand		0.13% Fe IOCS		0.22% Fe IOCS	
		EPM ($\mu\text{m cm s}^{-1}$ V^{-1})	ζ -potential (mV)	EPM ($\mu\text{m cm s}^{-1}$ V^{-1})	ζ -potential (mV)	EPM ($\mu\text{m cm s}^{-1}$ V^{-1})	ζ -potential (mV)
0.4 mM NaHCO_3 + 9.6 mM NaCl	8.5	-3.43 ± 0.16	-43.8 ± 2.1	-1.41 ± 0.09	-18.0 ± 1.2	-0.62 ± 0.04	-7.9 ± 0.6
0.4 mM NaHCO_3 + 3.2 mM CaCl_2	8.0	-0.95 ± 0.04	-12.1 ± 0.5	0.28 ± 0.03	3.6 ± 0.4	0.58 ± 8.35	7.3 ± 0.1
0.4 mM CH_3COONa + 2.6 mM CH_3COOH + 9.6 mM NaCl	3.8	-1.86 ± 0.09	-23.8 ± 1.2	1.35 ± 0.04	17.2 ± 0.5	2.96 ± 0.08	37.8 ± 1.0
0.4 mM CH_3COONa + 2.6 mM CH_3COOH + 3.2 mM CaCl_2	3.8	-0.70 ± 0.04	-8.9 ± 0.5	1.79 ± 0.04	22.8 ± 0.6	3.40 ± 0.15	43.4 ± 1.9

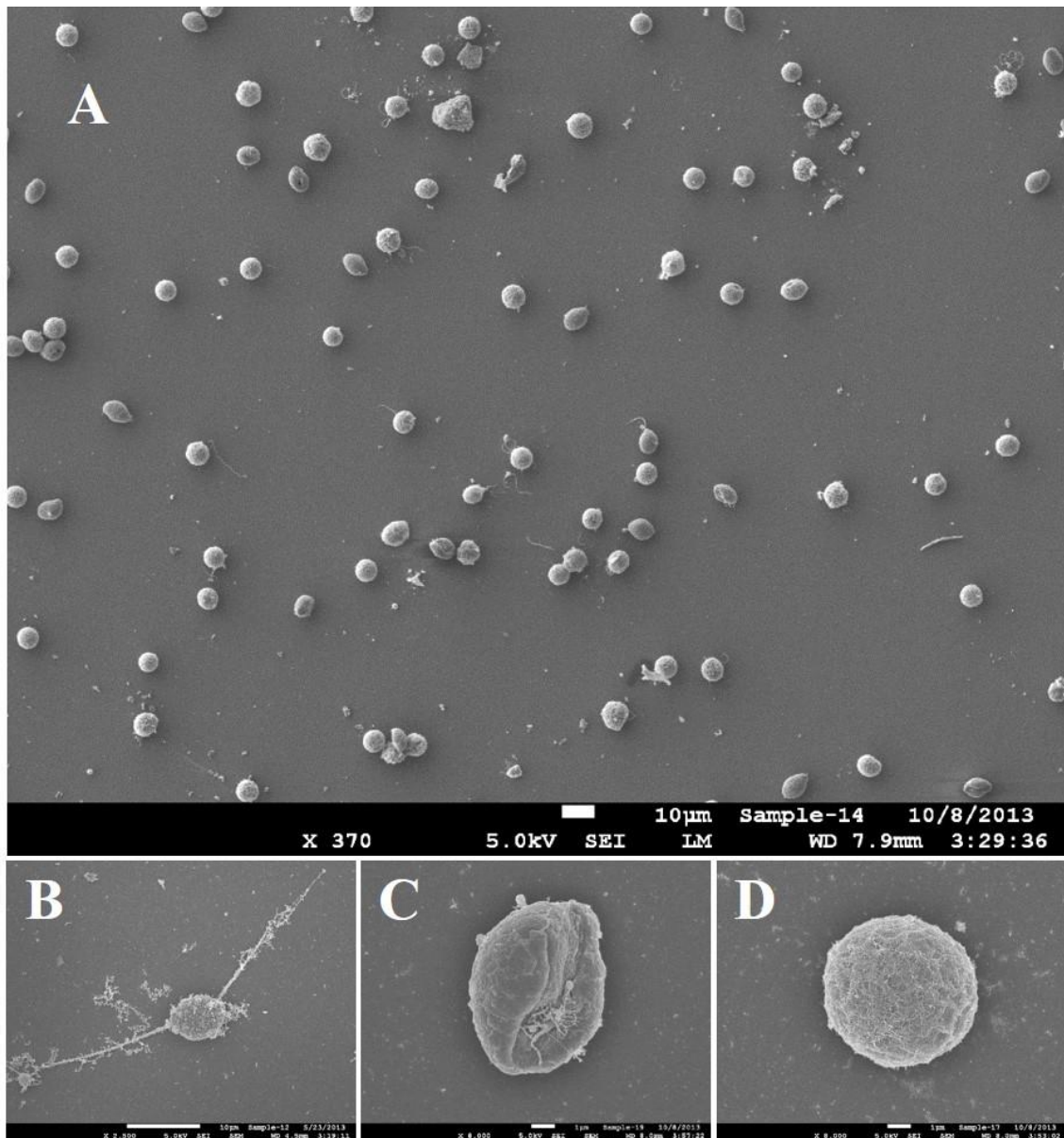


Figure 2.S6. SEM images of encysted (A, C and D) and biflagellate *P. capsici* zoospores (B) in deionized water.

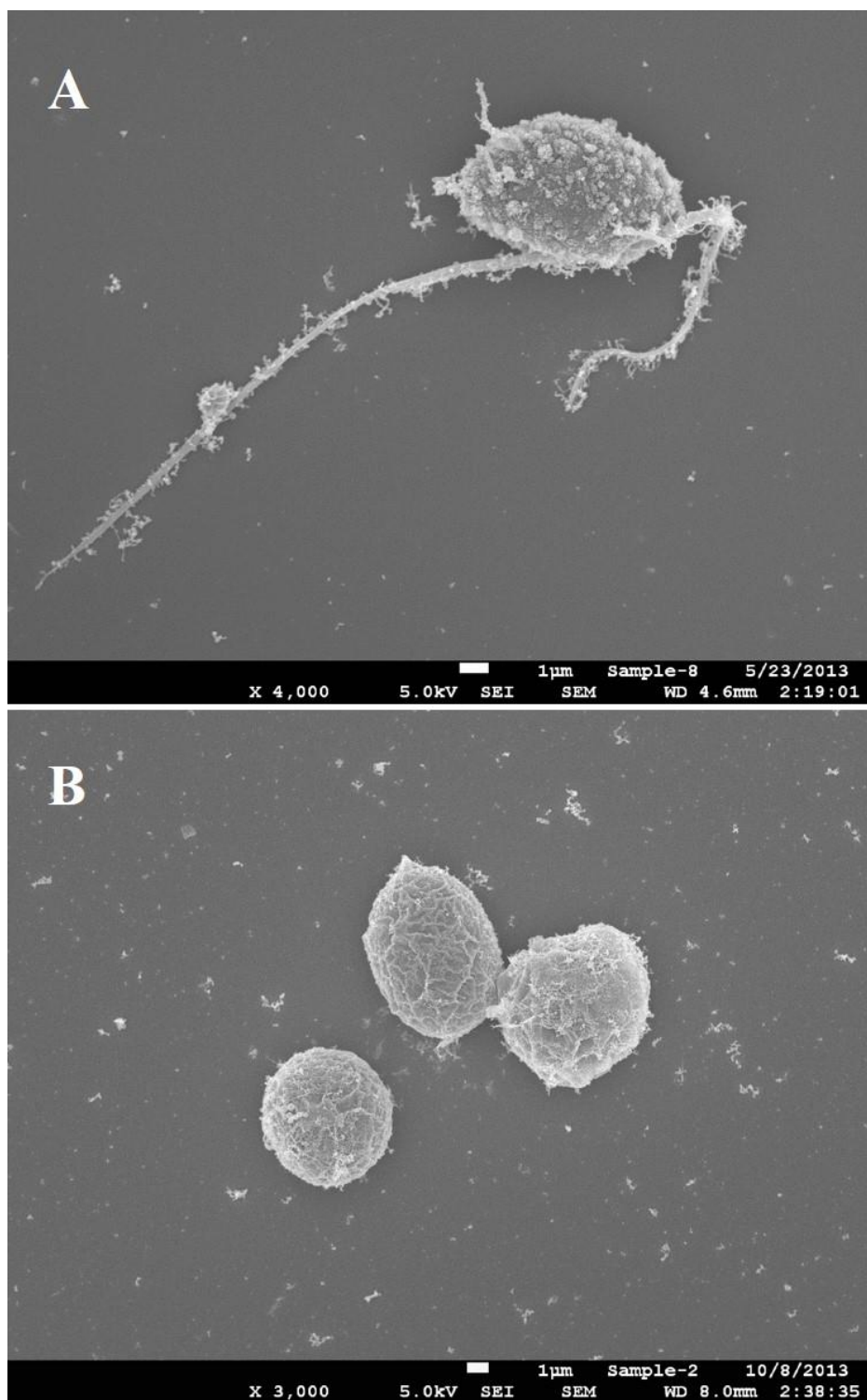


Figure 2.S7. SEM images of biflagellate (A) and encysted (B) *P. capsici* zoospores in NaCl solution at pH 7.2 and ionic strength 10 mM.

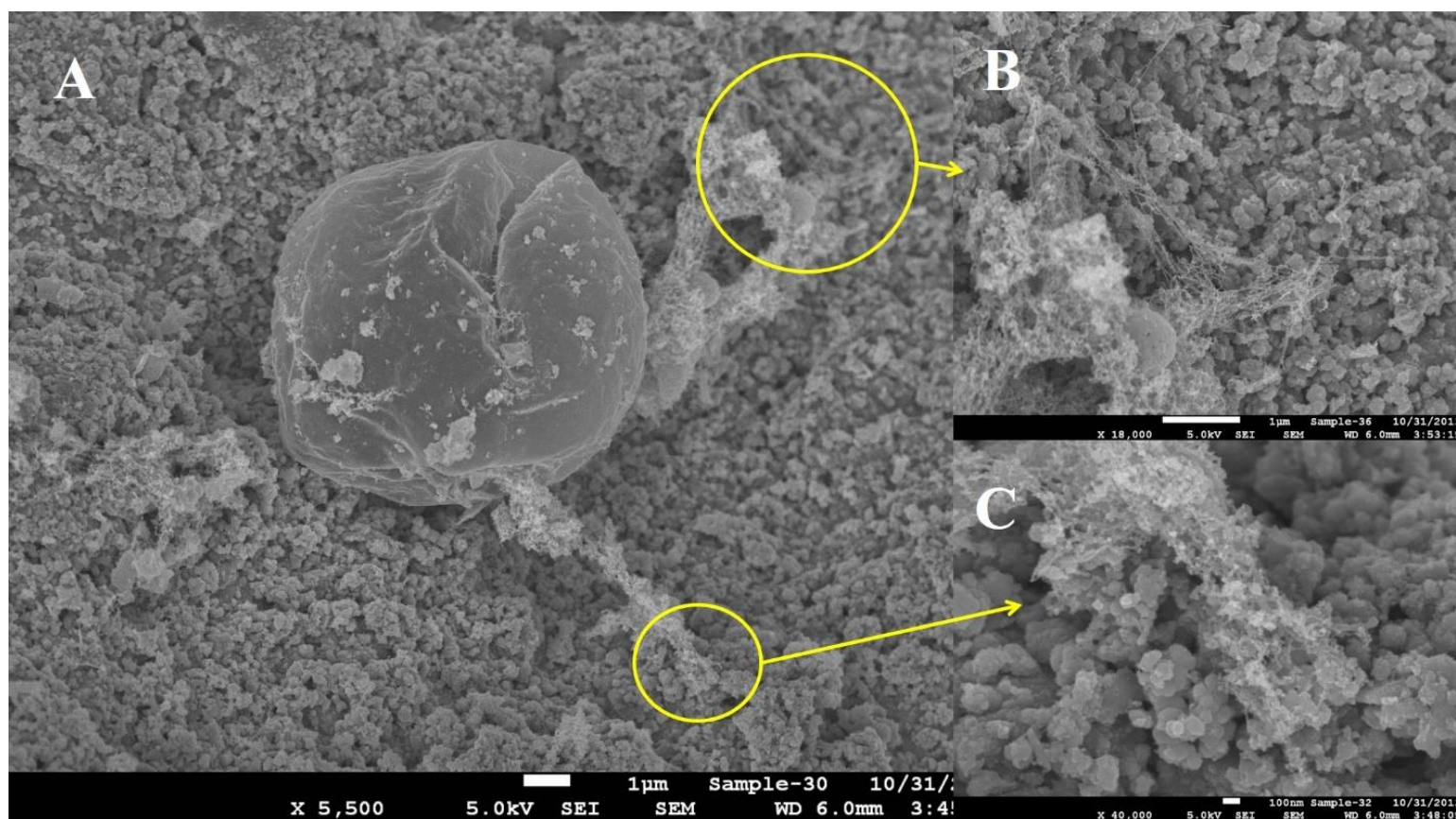


Figure 2.S8. SEM images of encysted *P. capsici* zoospores attached on iron oxide coated surface in the presence of Na^+ .

S2. XDLVO and α_{theory} and $k_{\text{d theory}}$ Calculations

The extended Derjaguin-Landau-Verwey-Overbeek (XDLVO) interaction energies, including Lifshitz-van der Waals (Φ^{LW}), electrical double layer (Φ^{EL}), and Born repulsion (Φ^{BR}) interactions, were calculated for a zoospore interacting with the solid-water interface (SWI), i.e., uncoated sand or IOCS surface. The total XDLVO interaction energy (Φ^{XDLVO}) was determined as a function of separation distance (x):

$$\Phi^{\text{XDLVO}}(x) = \Phi^{\text{LW}}(x) + \Phi^{\text{EL}}(x) + \Phi^{\text{BR}}(x) \quad (2.S1)$$

The Φ^{LW} , Φ^{EL} , and Φ^{BR} were calculated via:

$$\Phi^{\text{LW}}(x) = -\frac{A_{132}a_p}{6x}(1 + 14x/\lambda_c)^{-1} \quad (2.S2)^{86}$$

$$\Phi^{\text{EL}}(x) = \pi\epsilon\epsilon_0a_p \left\{ 2\psi_1\psi_2 \ln \left[\frac{1 + \exp(-\kappa x)}{1 - \exp(-\kappa x)} \right] + (\psi_1^2 + \psi_2^2) \ln[1 - \exp(-2\kappa x)] \right\} \quad (S3)^{87},$$

88

$$\Phi^{\text{BR}}(x) = \frac{A_{132}\sigma^6}{7560} \left[\frac{8a_p + x}{(2a_p + x)^7} + \frac{6a_p - x}{x^7} \right] \quad (2.S3)^{89}$$

where A_{132} is the Hamaker constant of the zoospore interacting with the SWI, a_p is the zoospore radius (i.e., 3.5 μm), λ_c is the van der Waals interaction characteristic wavelength (i.e., about 100 nm), ϵ is the dielectric constant of the medium (i.e., 80.1 for water at 293.15 K), ϵ_0 is the vacuum permittivity ($8.854 \times 10^{-12} \text{ C}^2 \text{ N}^{-1} \text{ m}^{-2}$), ψ_1 and ψ_2 are the respective surface potential of the zoospore and the SWI, κ is the reciprocal electric double layer thickness (the Debye length [κ^{-1}]), and σ (0.5 nm) is the collision diameter. The A_{132} value for the zoospore is unknown, therefore, the A_{132} of $6.5 \times 10^{-21} \text{ J}$ was taken from the literature values widely used for bacteria and *Cryptosporidium* oocysts.^{65, 90, 91} I used the ζ -potentials in place of the surface potential.⁹² The XDLVO

calculations were performed for the encysted zoospores. The ζ -potentials of the encysted zoospores in Table 2.1 under identical solution chemistry were averaged over several experimental sets, as shown in Table 2.S2. The ζ -potentials of the uncoated sand and IOCS of 0.13% Fe content as listed in Table 2.S2 were used to illustrate the XDLVO energy of the zoospore interacting with the sand and IOCS surfaces. The calculated XDLVO energies were normalized with kT where k is Boltzman constant (1.381×10^{-23} J K^{-1}) and T is temperature in Kelvin. The XDLVO primary energy maximum ($\Phi_{\max}$) and second energy minimum ($\Phi_{2\min}$) are listed in Table 2.S2 and the energy profiles are shown in Figure 2.S9. Theoretical attachment efficiency (α) is calculated from a Maxwell model that includes colloid deposition in secondary energy minimum.^{93, 94}

$$\alpha = 1 - \int_{\sqrt{\Phi_{2\min}}}^{\sqrt{\Delta\Phi}} \frac{4}{\pi^{1/2}} E^2 \exp(-E^2) dE \quad (2.S4)$$

where E^2 is the particle kinetic energy normalized by kT , and $\Delta\Phi$ is the sum of $\Phi_{\max}$ and $\Phi_{2\min}$. The calculated α values are provided in Table 2.S2 below.

Table 2.S2. XDLVO Calculations and Attachment Efficiency for an Encysted Zoospore Interacting with the Solid-Water Interface (SWI).^a

Zoospore-SWI	pH	Cation type	ζ -potential (mV)		$\Phi_{\max}$ (kT)	$\Phi_{2\min}$ (kT)	α
			Zoospore	SWI			
Zoospore-Sand	High pH (7.2–8.5)	Na ⁺	–18.0	–43.8	1770	6.6	0.996
Zoospore-Sand		Ca ⁺	–12.1	–12.1	137	12.0	1.000
Zoospore-IOCS		Na ⁺	–18.0	–18.0	675	8.7	0.999
Zoospore-IOCS		Ca ⁺	–12.1	3.6	<i>fav</i> ^b	<i>fav</i>	1.000
Zoospore-Sand	Low pH (3.8–4.4)	Na ⁺	–9.9	–23.8	301	9.8	1.000
Zoospore-Sand		Ca ⁺	–7.8	–8.9	<i>fav</i>	<i>fav</i>	1.000
Zoospore-IOCS		Na ⁺	–9.9	–17.2	<i>fav</i>	<i>fav</i>	1.000
Zoospore-IOCS		Ca ⁺	–7.8	–22.8	<i>fav</i>	<i>fav</i>	1.000

^a $\Phi_{\max}$ = primary energy maximum, $\Phi_{2\min}$ = second energy minimum, α_{theory} = theoretical attachment efficiency; ^b *fav* indicate the favorable condition with the absence of $\Phi_{\max}$.

As shown in Table 2.S2 and Figure 2.S9, at pH 7.2–8.5 the zoospore-sand interaction was characterized by high $\Phi_{\max}$ and appreciable $\Phi_{2\min}$, whereas the zoospore-

IOCS interaction had lower $\Phi_{\max}$ and deeper $\Phi_{2\min}$ in the presence of Na^+ , and no $\Phi_{\max}$ in the presence of Ca^{2+} . At pH 3.8–4.4, only the zoospore-sand interaction had $\Phi_{\max}$ and $\Phi_{2\min}$ in the presence of Na^+ , whereas all other interactions had no $\Phi_{\max}$. Because of either the sizable $\Phi_{2\min}$ or the absence of $\Phi_{\max}$, the calculated α was essentially 1, suggesting complete retention of the zoospores by the porous media.

Additionally, theoretical deposition rate coefficient ($k_{\text{d theory}}$) can be estimated as:⁹⁵

$$k_{\text{d theory}} = \frac{3}{2} \frac{(1-f)}{d_c} v \alpha \eta_0 \quad (2.S5)$$

where d_c is the effective collector diameter, f is the porosity (0.35), v is the pore water velocity (0.77 cm min^{-1}), and η_0 is the single-collector contact efficiency calculated by the Tufenkji and Elimelech equation.⁹⁵ The effective collector diameter was $375 \text{ }\mu\text{m}$ for the $250\text{--}500 \text{ }\mu\text{m}$ fraction and $652 \text{ }\mu\text{m}$ for the $500\text{--}804 \text{ }\mu\text{m}$ fraction, respectively. The zoospore density was 1.075 g cm^{-3} similar to *Cryptosporidium parvum* oocysts.⁶⁵ The calculated η_0 was $0.0275\text{--}0.0333$, and the $k_{\text{d theory}}$ was estimated to be $0.316\text{--}0.667$. Combining with Eq. (2), the theoretical effluent mass recovery (M_{ER}) was calculated to be $0\text{--}0.006$, again suggesting almost complete retention

S2. Column Breakthrough Experiments Raw Data ^a

Table 2.S3. Expt. 1: Biflagellate zoospores, IOCS, 500–804 μm , 0.11% Fe, pH 7.2 ± 0.2

Replicate 1				Replicate 2			
Na^+		Ca^{2+}		Na^+		Ca^{2+}	
$\theta = 0.35$		$\theta = 0.35$		$\theta = 0.35$		$\theta = 0.35$	
$v = 0.28 \text{ cm m}^{-1}$		$v = 0.27 \text{ cm m}^{-1}$		$v = 0.27 \text{ cm m}^{-1}$		$v = 0.8 \text{ cm m}^{-1}$	
Input PV = 1.8		Input PV = 1.9		Input PV = 1.9		Input PV = 1.8	
PV	C/C_0	PV	C/C_0	PV	C/C_0	PV	C/C_0
0.000	0.000	0.000	0.001	0.000	0.002	0.000	0.000
0.295	0.000	0.316	0.000	0.313	0.000	0.303	0.000
0.595	0.006	0.634	0.000	0.628	0.000	0.610	0.000
0.894	0.013	0.953	0.000	0.942	0.001	0.916	0.001
1.194	0.026	1.272	0.004	1.257	0.015	1.222	0.005
1.493	0.077	1.591	0.006	1.572	0.119	1.529	0.008
1.793	0.159	1.910	0.009	1.887	0.223	1.835	0.007
2.093	0.307	2.229	0.021	2.202	0.212	2.142	0.011
2.392	0.220	2.548	0.057	2.517	0.149	2.448	0.013
2.692	0.053	2.866	0.030	2.831	0.038	2.754	0.008
2.992	0.010	3.185	0.007	3.146	0.004	3.061	0.000
3.291	0.006	3.504	0.003	3.461	0.002	3.367	0.000
3.591	0.004	3.823	0.004	3.776	0.001	3.674	0.000
3.890	0.006	4.142	0.002	4.091	0.001	3.980	0.000
4.190	0.004	4.461	0.003	4.405	0.000	4.286	0.000
4.490	0.003	4.780	0.003	4.720	0.001	4.593	0.000
4.789	0.000	5.098	0.003	5.035	0.001	4.899	0.000
5.089	0.000	5.417	0.002	5.350	0.001	5.206	0.000
5.389	0.000	5.736	0.001	5.665	0.001	5.512	0.000
5.688	0.001	6.055	0.003	5.980	0.001	5.818	0.000
5.988	0.000	6.374	0.002	6.294	0.001	6.125	0.000
6.287	0.000	6.693	0.001	6.609	0.000	6.431	0.000
6.587	0.000	7.012	0.003	6.924	0.000	6.738	0.000
6.887	0.000	7.330	0.002	7.239	0.000	7.044	0.000

^a PV = Pore volumes, θ = volumetric water content, and v = average pore water velocity.

Table 2.S4. Expt. 2: Encysted zoospores, IOCS, 500–804 μm , 0.11% Fe, pH 7.2 ± 0.2

Replicate 1				Replicate 2			
Na^+		Ca^{2+}		Na^+		Ca^{2+}	
$\theta = 0.35$		$\theta = 0.35$		$\theta = 0.35$		$\theta = 0.35$	
$\nu = 0.28 \text{ cm m}^{-1}$		$\nu = 0.28 \text{ cm m}^{-1}$		$\nu = 0.28 \text{ cm m}^{-1}$		$\nu = 0.27 \text{ cm m}^{-1}$	
Input PV = 1.8		Input PV = 1.8		Input PV = 1.8		Input PV = 1.9	
PV	C/C_0	PV	C/C_0	PV	C/C_0	PV	C/C_0
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.298	0.002	0.295	0.000	0.296	0.001	0.302	0.000
0.601	0.000	0.595	0.000	0.597	0.003	0.610	0.002
0.903	0.014	0.895	0.007	0.898	0.009	0.918	0.006
1.206	0.027	1.195	0.016	1.198	0.011	1.226	0.005
1.509	0.029	1.495	0.016	1.499	0.016	1.534	0.008
1.811	0.032	1.795	0.015	1.800	0.014	1.842	0.009
2.114	0.033	2.095	0.014	2.101	0.023	2.150	0.010
2.417	0.027	2.395	0.015	2.402	0.025	2.458	0.011
2.719	0.010	2.695	0.006	2.702	0.019	2.766	0.004
3.022	0.002	2.995	0.006	3.003	0.012	3.074	0.001
3.325	0.003	3.295	0.000	3.304	0.009	3.382	0.002
3.627	0.006	3.595	0.000	3.605	0.008	3.690	0.001
3.930	0.002	3.895	0.000	3.905	0.008	3.998	0.000
4.233	0.000	4.195	0.000	4.206	0.016	4.306	0.001
4.535	0.000	4.495	0.000	4.507	0.009	4.614	0.000
4.838	0.002	4.795	0.000	4.808	0.009	4.922	0.000
5.141	0.002	5.094	0.000	5.108	0.009	5.230	0.001
5.444	0.000	5.394	0.000	5.409	0.002	5.538	0.002
5.746	0.000	5.694	0.000	5.710	0.004	5.846	0.000
6.049	0.000	5.994	0.000	6.011	0.005	6.154	0.000
6.352	0.000	6.294	0.000	6.312	0.006	6.462	0.000
6.654	0.000	6.594	0.000	6.612	0.005	6.770	0.002
6.957	0.000	6.894	0.000	6.913	0.007	7.078	0.003

continue

Table 2.S5. Expt. 2: Encysted zoospores, IOCS, 500–804 μm , 0.11% Fe, pH 7.2 ± 0.2

Replicate 3			
Na^+		Ca^{2+}	
$\theta = 0.35$		$\theta = 0.35$	
$\nu = 0.27 \text{ cm m}^{-1}$		$\nu = 0.26 \text{ cm m}^{-1}$	
Input PV = 1.9		Input PV = 1.9	
PV	C/C_0	PV	C/C_0
0.000	0.001	0.000	0.000
0.304	0.000	0.316	0.000
0.613	0.004	0.636	0.001
0.922	0.001	0.956	0.002
1.231	0.007	1.276	0.006
1.540	0.007	1.596	0.006
1.849	0.015	1.916	0.010
2.158	0.021	2.237	0.013
2.467	0.025	2.557	0.018
2.776	0.017	2.877	0.014
3.085	0.007	3.197	0.004
3.394	0.003	3.517	0.000
3.703	0.000	3.837	0.001
4.012	0.001	4.157	0.002
4.321	0.002	4.478	0.001
4.630	0.005	4.798	0.003
4.939	0.005	5.118	0.000
5.248	0.000	5.438	0.000
5.557	0.000	5.758	0.000
5.867	0.000	6.078	0.000
6.176	0.000	6.399	0.000
6.485	0.000	6.719	0.001
6.794	0.000	7.039	0.000
7.103	0.000	7.359	0.000

continue

Table 2.S6. Expt. 3: Encysted zoospores, Sand, 250~500 μm , 0% Fe, pH 7.2 ± 0.2

Replicate 1				Replicate 2			
Na^+		Ca^{2+}		Na^+		Ca^{2+}	
$\theta = 0.35$		$\theta = 0.36$		$\theta = 0.35$		$\theta = 0.36$	
$\nu = 0.28 \text{ cm m}^{-1}$		$\nu = 0.28 \text{ cm m}^{-1}$		$\nu = 0.27 \text{ cm m}^{-1}$		$\nu = 0.29 \text{ cm m}^{-1}$	
Input PV = 1.8		Input PV = 1.8		Input PV = 1.9		Input PV = 1.8	
PV	C/C_0	PV	C/C_0	PV	C/C_0	PV	C/C_0
0.000	0.001	0.000	0.000	0.000	0.002	0.000	0.000
0.296	0.000	0.288	0.000	0.315	0.000	0.286	0.000
0.596	0.000	0.581	0.001	0.633	0.000	0.578	0.000
0.896	0.007	0.875	0.013	0.952	0.015	0.869	0.008
1.197	0.045	1.168	0.054	1.270	0.057	1.160	0.049
1.497	0.051	1.462	0.061	1.588	0.061	1.451	0.060
1.798	0.054	1.755	0.063	1.907	0.064	1.743	0.058
2.098	0.054	2.049	0.065	2.225	0.065	2.034	0.061
2.398	0.057	2.342	0.067	2.544	0.068	2.325	0.063
2.699	0.042	2.635	0.050	2.862	0.046	2.616	0.045
2.999	0.004	2.929	0.011	3.181	0.014	2.908	0.007
3.300	0.006	3.222	0.010	3.499	0.003	3.199	0.000
3.600	0.003	3.516	0.009	3.817	0.004	3.490	0.001
3.900	0.005	3.809	0.000	4.136	0.004	3.782	0.001
4.201	0.003	4.103	0.002	4.454	0.000	4.073	0.001
4.501	0.004	4.396	0.003	4.773	0.002	4.364	0.000
4.802	0.002	4.689	0.005	5.091	0.002	4.655	0.000
5.102	0.000	4.983	0.002	5.410	0.002	4.947	0.000
5.402	0.000	5.276	0.003	5.728	0.003	5.238	0.001
5.703	0.000	5.570	0.002	6.046	0.004	5.529	0.001
6.003	0.000	5.863	0.003	6.365	0.003	5.820	0.001
6.304	0.000	6.157	0.003	6.683	0.000	6.112	0.002
6.604	0.000	6.450	0.004	7.002	0.002	6.403	0.001
6.904	0.000	6.743	0.003	7.320	0.000	6.694	0.001

continue

Table 2.S7. Expt. 3: Encysted zoospores, Sand, 250~500 μm , 0% Fe, pH 7.2 ± 0.2

Replicate 3			
Na^+		Ca^{2+}	
$\theta = 0.35$		$\theta = 0.36$	
$\nu = 0.28 \text{ cm m}^{-1}$		$\nu = 0.29 \text{ cm m}^{-1}$	
Input PV = 1.8		Input PV = 1.8	
PV	C/C_0	PV	C/C_0
0.000	0.000	0.000	0.000
0.297	0.000	0.290	0.000
0.598	0.001	0.583	0.002
0.898	0.007	0.876	0.003
1.199	0.039	1.170	0.033
1.500	0.041	1.463	0.049
1.800	0.042	1.757	0.049
2.101	0.042	2.050	0.044
2.402	0.048	2.343	0.043
2.703	0.035	2.637	0.019
3.003	0.011	2.930	0.004
3.304	0.010	3.224	0.000
3.605	0.005	3.517	0.000
3.906	0.007	3.810	0.000
4.206	0.005	4.104	0.000
4.507	0.004	4.397	0.000
4.808	0.004	4.691	0.000
5.108	0.004	4.984	0.000
5.409	0.000	5.277	0.000
5.710	0.000	5.571	0.000
6.011	0.000	5.864	0.000
6.311	0.000	6.158	0.000
6.612	0.000	6.451	0.000
6.913	0.000	6.744	0.000

continue

Table 2.S 8. Expt. 4: Encysted zoospores, IOCS,250~500 μm , 0.13% Fe, pH 7.2 ± 0.2

Replicate 1				Replicate 2			
Na^+		Ca^{2+}		Na^+		Ca^{2+}	
$\theta = 0.36$		$\theta = 0.36$		$\theta = 0.36$		$\theta = 0.36$	
$\nu = 0.28 \text{ cm m}^{-1}$		$\nu = 0.29 \text{ cm m}^{-1}$		$\nu = 0.28 \text{ cm m}^{-1}$		$\nu = 0.28 \text{ cm m}^{-1}$	
Input PV = 1.8		Input PV = 1.8		Input PV = 1.8		Input PV = 2.0	
PV	C/C_0	PV	C/C_0	PV	C/C_0	PV	C/C_0
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.296	0.001	0.290	0.000	0.293	0.002	0.343	0.000
0.597	0.002	0.584	0.000	0.590	0.004	0.675	0.000
0.897	0.002	0.878	0.000	0.887	0.004	1.007	0.000
1.197	0.002	1.171	0.003	1.184	0.007	1.339	0.005
1.497	0.015	1.465	0.018	1.480	0.019	1.670	0.015
1.798	0.026	1.759	0.029	1.777	0.030	2.002	0.030
2.098	0.035	2.053	0.034	2.074	0.036	2.334	0.040
2.398	0.035	2.347	0.039	2.371	0.036	2.666	0.027
2.698	0.022	2.641	0.037	2.667	0.028	2.997	0.005
2.998	0.000	2.935	0.017	2.964	0.011	3.329	0.000
3.299	0.000	3.229	0.007	3.261	0.003	3.661	0.000
3.599	0.000	3.523	0.004	3.558	0.005	3.993	0.000
3.899	0.007	3.816	0.003	3.854	0.004	4.325	0.000
4.199	0.007	4.110	0.005	4.151	0.005	4.656	0.000
4.499	0.000	4.404	0.000	4.448	0.004	4.988	0.000
4.800	0.000	4.698	0.000	4.745	0.004	5.320	0.000
5.100	0.000	4.992	0.000	5.041	0.007	5.652	0.000
5.400	0.000	5.286	0.000	5.338	0.006	5.983	0.000
5.700	0.000	5.580	0.000	5.635	0.003	6.315	0.000
6.001	0.000	5.874	0.000	5.932	0.003	6.647	0.000
6.301	0.000	6.168	0.000	6.229	0.001	6.979	0.000
6.601	0.000	6.462	0.000	6.525	0.003	7.310	0.000
6.901	0.000	6.755	0.000	6.822	0.003	7.642	0.000

continue

Table 2.S 9. Expt. 4: Encysted zoospores, IOCS, 250~500 μm , 0.13% Fe, pH 7.2 ± 0.2

Replicate 3			
Na^+		Ca^{2+}	
$\theta = 0.34$		$\theta = 0.34$	
$\nu = 0.27 \text{ cm m}^{-1}$		$\nu = 0.26 \text{ cm m}^{-1}$	
Input PV = 1.9		Input PV = 1.9	
PV	C/C_0	PV	C/C_0
0.000	0.000	0.000	0.000
0.305	0.001	0.316	0.000
0.616	0.000	0.636	0.000
0.927	0.000	0.956	0.000
1.237	0.001	1.276	0.000
1.548	0.008	1.595	0.010
1.859	0.016	1.915	0.016
2.170	0.019	2.235	0.017
2.480	0.021	2.555	0.022
2.791	0.012	2.874	0.014
3.102	0.002	3.194	0.004
3.413	0.000	3.514	0.001
3.723	0.000	3.834	0.001
4.034	0.000	4.153	0.000
4.345	0.000	4.473	0.000
4.656	0.000	4.793	0.000
4.966	0.000	5.113	0.000
5.277	0.000	5.432	0.000
5.588	0.000	5.752	0.004
5.899	0.000	6.072	0.004
6.209	0.002	6.392	0.000
6.520	0.000	6.711	0.002
6.831	0.000	7.031	0.003
7.142	0.000	7.351	0.000

continue

Table 2.S 10. Expt. 5: Encysted zoospores, IOCS, 250~500 μm , 0.22% Fe, pH 7.2 ± 0.2

Replicate 1				Replicate 2			
Na^+		Ca^{2+}		Na^+		Ca^{2+}	
$\theta = 0.35$		$\theta = 0.35$		$\theta = 0.35$		$\theta = 0.35$	
$\nu = 0.23 \text{ cm m}^{-1}$		$\nu = 0.27 \text{ cm m}^{-1}$		$\nu = 0.26 \text{ cm m}^{-1}$		$\nu = 0.28 \text{ cm m}^{-1}$	
Input PV = 1.9		Input PV = 1.9		Input PV = 2.0		Input PV = 1.8	
PV	C/C_0	PV	C/C_0	PV	C/C_0	PV	C/C_0
0.000	0.001	0.000	0.003	0.000	0.000	0.000	0.000
0.355	0.000	0.305	0.000	0.324	0.000	0.296	0.000
0.716	0.000	0.615	0.000	0.652	0.003	0.597	0.001
1.076	0.000	0.924	0.000	0.980	0.000	0.898	0.000
1.437	0.002	1.234	0.003	1.308	0.000	1.198	0.000
1.797	0.005	1.544	0.005	1.636	0.001	1.499	0.001
2.158	0.010	1.854	0.007	1.964	0.004	1.800	0.001
2.519	0.011	2.163	0.003	2.292	0.009	2.100	0.001
2.879	0.005	2.473	0.005	2.620	0.014	2.401	0.006
3.240	0.003	2.783	0.001	2.948	0.013	2.702	0.009
3.600	0.005	3.092	0.004	3.276	0.006	3.002	0.002
3.961	0.019	3.402	0.000	3.604	0.005	3.303	0.001
4.322	0.000	3.712	0.002	3.931	0.002	3.604	0.000
4.682	0.000	4.022	0.005	4.259	0.001	3.904	0.000
5.043	0.004	4.331	0.008	4.587	0.000	4.205	0.000
5.403	0.001	4.641	0.003	4.915	0.000	4.506	0.000
5.764	0.001	4.951	0.003	5.243	0.000	4.806	0.001
6.125	0.005	5.260	0.004	5.571	0.000	5.107	0.000
6.485	0.000	5.570	0.003	5.899	0.000	5.408	0.000
6.846	0.000	5.880	0.003	6.227	0.000	5.708	0.000
7.206	0.000	6.190	0.002	6.555	0.000	6.009	0.000
7.567	0.000	6.499	0.002	6.883	0.000	6.310	0.000
7.928	0.000	6.809	0.003	7.211	0.000	6.611	0.000
8.288	0.000	7.119	0.003	7.539	0.000	6.911	0.000

continue

Table 2.S 11. Expt. 6: Encysted zoospores, Sand, 500~804 μm , 0% Fe, pH 7.2 ± 0.2

Replicate 1				Replicate 2			
Na^+		Ca^{2+}		Na^+		Ca^{2+}	
$\theta = 0.35$		$\theta = 0.35$		$\theta = 0.35$		$\theta = 0.35$	
$\nu = 0.28 \text{ cm m}^{-1}$		$\nu = 0.25 \text{ cm m}^{-1}$		$\nu = 0.26 \text{ cm m}^{-1}$		$\nu = 0.27 \text{ cm m}^{-1}$	
Input PV = 1.8		Input PV = 2.0		Input PV = 2.0		Input PV = 1.9	
PV	C/C_0	PV	C/C_0	PV	C/C_0	PV	C/C_0
0.000	0.001	0.000	0.002	0.000	0.000	0.000	0.001
0.301	0.000	0.324	0.000	0.317	0.001	0.304	0.000
0.606	0.000	0.653	0.000	0.640	0.001	0.614	0.001
0.911	0.017	0.982	0.009	0.964	0.016	0.924	0.009
1.216	0.046	1.311	0.038	1.288	0.043	1.234	0.031
1.521	0.056	1.640	0.049	1.612	0.052	1.544	0.041
1.826	0.052	1.969	0.047	1.935	0.049	1.854	0.049
2.131	0.053	2.298	0.053	2.259	0.049	2.164	0.048
2.436	0.051	2.628	0.051	2.583	0.047	2.474	0.048
2.741	0.035	2.957	0.037	2.907	0.031	2.784	0.034
3.046	0.012	3.286	0.006	3.231	0.001	3.094	0.013
3.351	0.001	3.615	0.000	3.554	0.000	3.404	0.008
3.656	0.000	3.944	0.000	3.878	0.000	3.714	0.008
3.961	0.000	4.273	0.000	4.202	0.000	4.024	0.006
4.266	0.000	4.602	0.000	4.526	0.000	4.334	0.008
4.571	0.000	4.931	0.000	4.849	0.000	4.644	0.002
4.876	0.000	5.261	0.000	5.173	0.000	4.954	0.003
5.181	0.000	5.590	0.000	5.497	0.000	5.264	0.002
5.486	0.000	5.919	0.001	5.821	0.000	5.575	0.002
5.791	0.000	6.248	0.001	6.144	0.000	5.885	0.001
6.096	0.000	6.577	0.000	6.468	0.000	6.195	0.002
6.401	0.000	6.906	0.001	6.792	0.000	6.505	0.001
6.706	0.000	7.235	0.000	7.116	0.000	6.815	0.002
7.011	0.000	7.564	0.000	7.440	0.000	7.125	0.000

continue

Table 2.S 12. Expt. 6: Encysted zoospores, Sand, 500~804 μm , 0% Fe, pH 7.2 ± 0.2

Replicate 3			
Na^+		Ca^{2+}	
$\theta = 0.35$		$\theta = 0.36$	
$\nu = 0.25 \text{ cm m}^{-1}$		$\nu = 0.28 \text{ cm m}^{-1}$	
Input PV = 2.0		Input PV = 1.9	
PV	C/C_0	PV	C/C_0
0.000	0.000	0.000	0.000
0.326	0.002	0.319	0.000
0.658	0.000	0.636	0.007
0.989	0.007	0.952	0.003
1.320	0.020	1.269	0.016
1.651	0.027	1.586	0.026
1.982	0.023	1.903	0.024
2.313	0.023	2.219	0.028
2.645	0.022	2.536	0.022
2.976	0.013	2.853	0.019
3.307	0.000	3.170	0.002
3.638	0.000	3.486	0.000
3.969	0.000	3.803	0.000
4.301	0.000	4.120	0.000
4.632	0.000	4.437	0.000
4.963	0.000	4.753	0.000
5.294	0.000	5.070	0.000
5.625	0.000	5.387	0.000
5.956	0.000	5.704	0.000
6.288	0.000	6.020	0.000
6.619	0.000	6.337	0.000
6.950	0.000	6.654	0.000
7.281	0.000	6.971	0.000
7.612	0.000	7.287	0.000

continue

Table 2.S 13. Expt. 7: Encysted zoospores, Sand, 250~500 μm , 0% Fe, pH 4.4 ± 0.1

Replicate 1			
Na^+		Ca^{2+}	
$\theta = 0.34$		$\theta = 0.35$	
$\nu = 0.28 \text{ cm m}^{-1}$		$\nu = 0.27 \text{ cm m}^{-1}$	
Input PV = 1.8		Input PV = 1.9	
PV	C/C_0	PV	C/C_0
0.000	0.000	0.000	0.000
0.301	0.001	0.308	0.002
0.606	0.008	0.620	0.001
0.911	0.006	0.932	0.000
1.217	0.003	1.244	0.003
1.522	0.012	1.556	0.004
1.827	0.009	1.869	0.006
2.133	0.004	2.181	0.003
2.438	0.004	2.493	0.007
2.743	0.007	2.805	0.012
3.049	0.008	3.118	0.000
3.354	0.000	3.430	0.000
3.659	0.003	3.742	0.000
3.964	0.001	4.054	0.000
4.270	0.000	4.366	0.000
4.575	0.005	4.679	0.000
4.880	0.009	4.991	0.000
5.186	0.003	5.303	0.000
5.491	0.006	5.615	0.000
5.796	0.006	5.927	0.000
6.101	0.006	6.240	0.000
6.407	0.005	6.552	0.000
6.712	0.004	6.864	0.000
7.017	0.007	7.176	0.000

continue

Table 2.S 14. Tracer(NaBr), sand 250~500 μm , IS = 0, $C_0 = 100 \text{ mg L}^{-1}$

$\theta = 0.35$	
$\nu = 0.28 \text{ cm m}^{-1}$	
Input PV = 1.7	
PV	C/C_0
0.000	0.001
0.276	0.000
0.564	0.000
0.851	0.157
1.139	0.959
1.426	1.015
1.714	1.007
2.001	1.009
2.289	1.019
2.576	0.927
2.864	0.106
3.152	0.004
3.439	0.002
3.727	0.000
4.014	0.000
4.302	0.000
4.589	0.000
4.877	0.000
5.164	0.000
5.452	0.000
5.739	0.000
6.027	0.000
6.314	0.000
6.602	0.000

continue

Table 2.S 15. Expt. 8: Encysted zoospores, IOCS, 250~500 μm , 0.13% Fe, pH 4.4 ± 0.1

Replicate 1			
Na^+		Ca^{2+}	
$\theta = 0.34$		$\theta = 0.35$	
$\nu = 0.27 \text{ cm m}^{-1}$		$\nu = 0.27 \text{ cm m}^{-1}$	
Input PV = 1.8		Input PV = 1.8	
PV	C/C_0	PV	C/C_0
0.000	0.000	0.000	0.000
0.298	0.008	0.301	0.000
0.601	0.006	0.606	0.000
0.905	0.000	0.912	0.005
1.208	0.000	1.217	0.002
1.511	0.005	1.523	0.003
1.815	0.003	1.828	0.000
2.118	0.003	2.134	0.000
2.422	0.000	2.440	0.000
2.725	0.001	2.745	0.000
3.029	0.000	3.051	0.000
3.332	0.000	3.356	0.000
3.635	0.000	3.662	0.000
3.939	0.000	3.968	0.000
4.242	0.000	4.273	0.000
4.546	0.000	4.579	0.000
4.849	0.000	4.884	0.000
5.152	0.000	5.190	0.001
5.456	0.000	5.495	0.000
5.759	0.000	5.801	0.000
6.063	0.000	6.107	0.000
6.366	0.000	6.412	0.000
6.669	0.000	6.718	0.000
6.973	0.000	7.023	0.000

continue

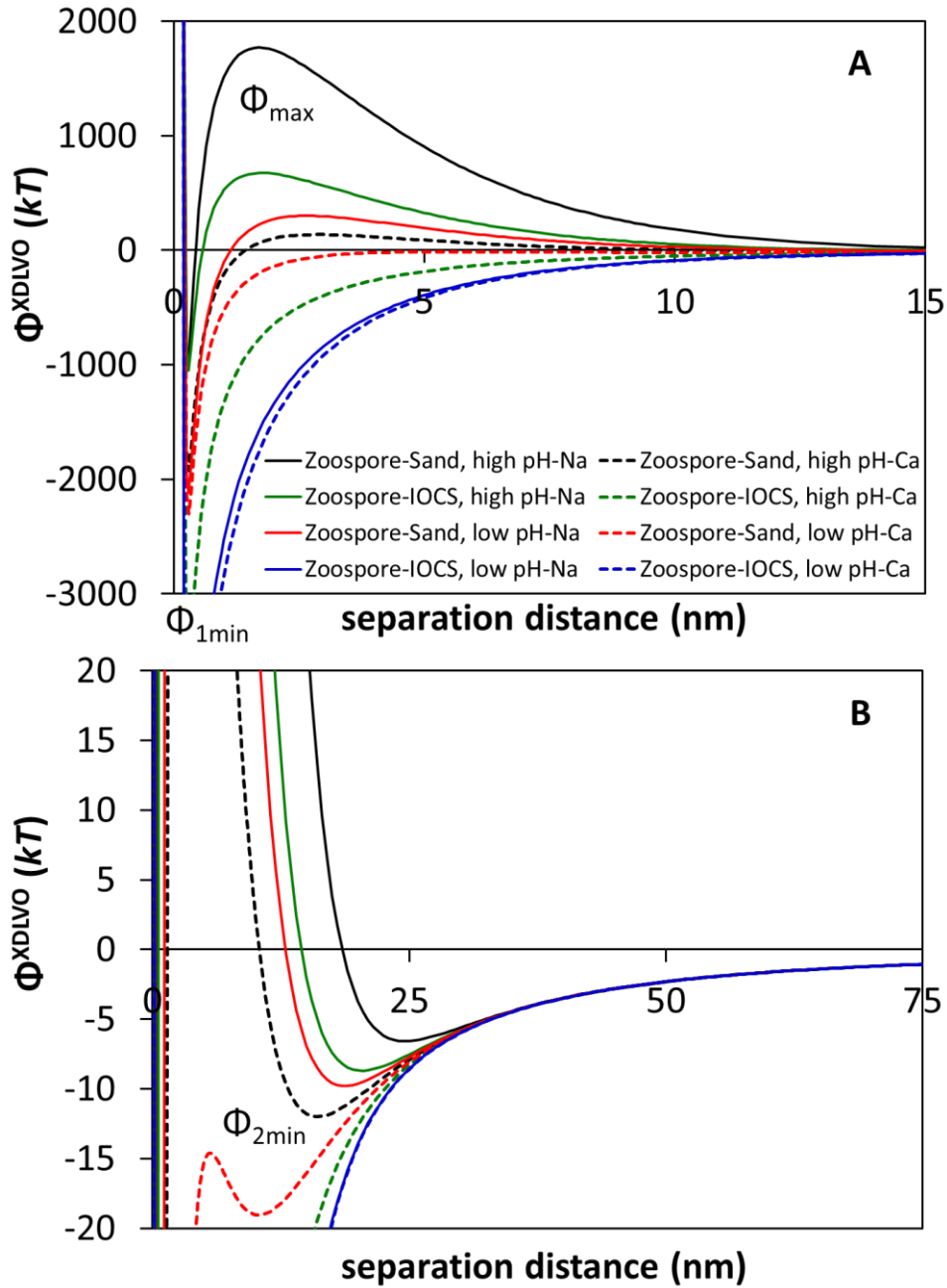


Figure 2.S9. Total XDLVO interaction energies of zoospore interacting with uncoated sand surface or iron oxide coated sand (IOCS) surface: (a) primary energy minimum (Φ_{1min}) and maximum (Φ_{max}) and (b) secondary energy minimum (Φ_{2min}).

REFERENCES

REFERENCES

1. Pimentel, D.; McLaughlin, L.; Zepp, A.; Lakitan, B.; Kraus, T.; Kleinman, P.; Vancini, F.; Roach, W. J.; Graap, E.; Keeton, W. S.; Selig, G., Environmental and economic effects of reducing pesticide use in agriculture. *Agric., Ecosyst. Environ.* **1993**, *46*, (1–4), 273-288.
2. Pimentel, D.; McNair, S.; Janecka, J.; Wightman, J.; Simmonds, C.; O’Connell, C.; Wong, E.; Russel, L.; Zern, J.; Aquino, T.; Tsomondo, T., Economic and environmental threats of alien plant, animal, and microbe invasions. *Agric., Ecosyst. Environ.* **2001**, *84*, (1), 1-20.
3. Ochiai, N. Movement of zoospores of *Phytophthora citricola* in saturated porous media. Ph.D. Dissertation, Oregon State University, Corvallis, 2010.
4. Bradford, S. A.; Morales, V. L.; Zhang, W.; Harvey, R. W.; Packman, A. I.; Mohanram, A.; Welty, C., Transport and fate of microbial pathogens in agricultural settings. *Crit. Rev. Env. Sci. Technol.* **2013**, *43*, (8), 775-893.
5. McDowell-Boyer, L. M.; Hunt, J. R.; Sitar, N., Particle transport through porous media. *Water Resour. Res.* **1986**, *22*, (13), 1901-1921.
6. Jin, Y.; Flury, M., Fate and transport of viruses in porous media. In *Advances in Agronomy*, Academic Press: 2002; Vol. Volume 77, pp 39-102.
7. Ginn, T. R.; Wood, B. D.; Nelson, K. E.; Scheibe, T. D.; Murphy, E. M.; Clement, T. P., Processes in microbial transport in the natural subsurface. *Advances in Water Resources* **2002**, *25*, (8-12), 1017-1042.
8. Granke, L. L.; Quesada-Ocampo, L.; Lamour, K.; Hausbeck, M. K., Advances in Research on *Phytophthora capsici* on Vegetable Crops in The United States. *Plant Dis.* **2012**, *96*, (11), 1588-1600.
9. Hausbeck, M. K.; Lamour, K. H., *Phytophthora capsici* on Vegetable Crops: Research Progress and Management Challenges. *Plant Dis.* **2004**, *88*, (12), 1292-1303.
10. Sanogo, S.; Ji, P., Water management in relation to control of *Phytophthora capsici* in vegetable crops. *Agric. Water Manage.* **2013**, *129*, (0), 113-119.
11. Hong, C. X.; Moorman, G. W., Plant pathogens in irrigation water: Challenges and opportunities. *Crit. Rev. Plant Sci.* **2005**, *24*, (3), 189-208.
12. Sanogo, S.; Ji, P., Integrated management of *Phytophthora capsici* on solanaceous and cucurbitaceous crops: current status, gaps in knowledge and research needs.

13. Gevens, A.; Donahoo, R.; Lamour, K.; Hausbeck, M., Characterization of *Phytophthora capsici* from Michigan surface irrigation water. *Phytopathology* **2007**, 97, (4), 421-428.
14. Lewis Ivey, M. L.; Miller, S. A., Assessing the efficacy of pre-harvest, chlorine-based sanitizers against human pathogen indicator microorganisms and *Phytophthora capsici* in non-recycled surface irrigation water. *Water Res.* **2013**, 47, (13), 4639-4651.
15. Martínez, F.; Castilla, S.; Tello, J. C.; Avilés, M., Spread of *Phytophthora cactorum* and *Verticillium dahliae* by slow sand filtration treatment of the recirculating nutrient solution on closed soilless growing systems in strawberry. *ISHS Acta Horticulturae: International Symposium on Soilless Culture and Hydroponics* **2005**, 697, 411-416.
16. Bush, E. A.; Hong, C.; Stromberg, E. L., Fluctuations of *Phytophthora* and *Pythium* spp. in components of a recycling irrigation system. *Plant Dis.* **2003**, 87, (12), 1500-1506.
17. Hong, C.; Richardson, P.; Kong, P.; Bush, E., Efficacy of chlorine on multiple species of *Phytophthora* in recycled nursery irrigation water. *Plant Dis* **2003**, 87, (10), 1183-1189.
18. Meador, D. P.; Fisher, P. R.; Harmon, P. F.; Peres, N. A.; Teplitzki, M.; Guy, C. L., Survey of physical, chemical, and microbial water quality in greenhouse and nursery irrigation water. *HortTechnology* **2012**, 22, (6), 778-786.
19. Raudales, R. E.; Parke, J. L.; Guy, C. L.; Fisher, P. R., Control of waterborne microbes in irrigation: A review. *Agric. Water Manage.* **2014**, 143, (0), 9-28.
20. Raudales, R. E.; Irani, T. A.; Hall, C. R.; Fisher, P. R., Modified Delphi survey on key attributes for selection of water-treatment technologies for horticulture irrigation. *HortTechnology* **2014**, 24, (3), 355-368.
21. Lamour, K. H.; Hausbeck, M. K., Susceptibility of Mefenoxam-Treated Cucurbits to Isolates of *Phytophthora capsici* Sensitive and Insensitive to Mefenoxam. *Plant Dis.* **2003**, 87, (8), 920-922.
22. Lu, X. H.; Hausbeck, M. K.; Liu, X. L.; Hao, J. J., Wild Type Sensitivity and Mutation Analysis for Resistance Risk to Fluopicolide in *Phytophthora capsici*. *Plant Dis.* **2011**, 95, (12), 1535-1541.
23. Martínez, F.; Castillo, S.; Carmona, E.; Avilés, M., Dissemination of *Phytophthora cactorum*, cause of crown rot in strawberry, in open and closed soilless growing systems and the potential for control using slow sand filtration. *Scientia Horticulturae* **2010**, 125, (4), 756-760.

24. van Os, E. A.; van Kuik, F. J.; Runia, W. T. h.; van Buuren, J., Prospects of slow sand filtration to eliminate pathogens from recirculating nutrient solutions. *ISHS Acta Horticulturae* **1998**, 458, (377-384).
25. Calvo-Bado, L. A.; Pettitt, T. R.; Parsons, N.; Petch, G. M.; Morgan, J. A. W.; Whipps, J. M., Spatial and temporal analysis of the microbial community in slow sand filters used for treating horticultural irrigation water. *Appl. Environ. Microbiol.* **2003**, 69, (4), 2116-2125.
26. Wilkinson, H. T.; Miller, R. D.; Millar, R. L., Infiltration of Fungal and Bacterial Propagules into Soil. *Soil Sci Soc Am J* **1981**, 45, (6), 1034-1039.
27. Benjamin, M.; Newhook, F. J., Effect of glass microbeads on *Phytophthora* zoospore motility. *Transactions of the British Mycological Society* **1982**, 78, (1), 43-46.
28. Duniway, J. M., Movement of zoospores of *Phytophthora cryptogea* in soils of various textures and matric potentials. *Phytopathology* **1976**, 66, (877-882).
29. Young, B. R.; Newhook, F. J.; Allen, R. N., Motility and chemotactic response of *Phytophthora cinnamomi* zoospores in 'ideal soils'. *Transactions of the British Mycological Society* **1979**, 72, (3), 395-401.
30. Ho, H. H.; Hickman, C. J., Asexual Reproduction and behavior of zoospores of *Phytophthora megasperma* var. *sojae*. *Can. J. Bot.* **1967**, 45, (11), 1963-1981.
31. Newhook, F. J.; Young, B. R.; Allen, S. D.; Allen, R. N., Zoospore motility of *Phytophthora cinnamomi* in particulate substrates. *J. Phytopathol.* **1981**, 101, (3), 202-209.
32. Bimpong, C. E.; Hickman, C. J., Ultrastructural and cytochemical studies of zoospores, cysts, and germinating cysts of *Phytophthora palmivora*. *Can. J. Bot.* **1975**, 53, (13), 1310-1327.
33. Hemmes, D. E.; Hohl, H. R., Ultrastructural Aspects of Encystation and Cyst-Germination in *Phytophthora Parasitica*. *J. Cell Sci.* **1971**, 9, (1), 175-191.
34. Xu, S.; Saiers, J. E., Colloid straining within water-saturated porous media: Effects of colloid size nonuniformity. *Water Resour. Res.* **2009**, 45, (5), W05501.
35. Tong, M.; Johnson, W. P., Excess colloid retention in porous media as a function of colloid size, fluid velocity, and grain angularity. *Environ. Sci. Technol.* **2006**, 40, (24), 7725-7731.
36. Xu, S.; Liao, Q.; Saiers, J. E., Straining of nonspherical colloids in saturated porous media. *Environ. Sci. Technol.* **2008**, 42, (3), 771-778.
37. Xu, S.; Gao, B.; Saiers, J. E., Straining of colloidal particles in saturated porous

- media. *Water Resour. Res.* **2006**, 42, (12), W12S16.
38. Gevens, A. J.; Donahoo, R. S.; Lamour, K. H.; Hausbeck, M. K., Characterization of *Phytophthora capsici* causing foliar and pod blight of snap bean in Michigan. *Plant Dis.* **2008**, 92, (2), 201-209.
 39. Bradford, S. A.; Kim, H., Implications of cation exchange on clay release and colloid-facilitated transport in porous media. *J. Environ. Qual.* **2010**, 39, (6), 2040-2046.
 40. Schwertmann, U.; Cornell, R. M., Goethite. In *Iron Oxides in the Laboratory*, Wiley-VCH Verlag GmbH: 2000; pp 67-92.
 41. Zhang, W.; Niu, J.; Morales, V. L.; Chen, X.; Hay, A. G.; Lehmann, J.; Steenhuis, T. S., Transport and retention of biochar particles in porous media: effect of pH, ionic strength, and particle size. *Ecohydrology* **2010**, 3, (4), 497-508.
 42. Johnson, P. R., A comparison of streaming and microelectrophoresis methods for obtaining the ζ potential of granular porous media surfaces. *J. Colloid Interface Sci.* **1999**, 209, (1), 264-267.
 43. Akbour, R. A.; Douch, J.; Hamdani, M.; Schmitz, P., Transport of kaolinite colloids through quartz sand: Influence of humic acid, Ca^{2+} , and trace metals. *J. Colloid Interface Sci.* **2002**, 253, (1), 1-8.
 44. Schweich, D.; Sardin, M., Adsorption, partition, ion exchange and chemical reaction in batch reactors or in columns — A review. *Journal of Hydrology* **1981**, 50, (0), 1-33.
 45. Kretzschmar, R.; Barmettler, K.; Grolimund, D.; Yan, Y.-d.; Borkovec, M.; Sticher, H., Experimental determination of colloid deposition rates and collision efficiencies in natural porous media. *Water Resour. Res.* **1997**, 33, (5), 1129-1137.
 46. Zhang, W.; Morales, V. L.; Cakmak, M. E.; Salvucci, A. E.; Geohring, L. D.; Hay, A. G.; Parlange, J.-Y.; Steenhuis, T. S., Colloid transport and retention in unsaturated porous media: Effect of colloid input concentration. *Environ. Sci. Technol.* **2010**, 44, (13), 4965-4972.
 47. Mukherjee, B.; Weaver, J. W., Aggregation and charge behavior of metallic and nonmetallic nanoparticles in the presence of competing similarly-charged inorganic ions. *Environ. Sci. Technol.* **2010**, 44, (9), 3332-3338.
 48. Brant, J.; Lecoanet, H.; Hotze, M.; Wiesner, M., Comparison of electrokinetic properties of colloidal fullerenes (n-C₆₀) formed using two procedures. *Environ. Sci. Technol.* **2005**, 39, (17), 6343-6351.
 49. Sang, W.; Stoof, C. R.; Zhang, W.; Morales, V. L.; Gao, B.; Kay, R. W.; Liu, L.; Zhang, Y.; Steenhuis, T. S., Effect of hydrofracking fluid on colloid transport in the

unsaturated zone. *Environ. Sci. Technol.* **2014**, 48, (14), 8266-8274.

50. Zhang, W.; Isaacson, C. W.; Rattanaudompol, U.; Powell, T. B.; Bouchard, D., Fullerene nanoparticles exhibit greater retention in freshwater sediment than in model porous media. *Water Res.* **2012**, 46, (9), 2992-3004.
51. Abudalo, R. A.; Bogatsu, Y. G.; Ryan, J. N.; Harvey, R. W.; Metge, D. W.; Elimelech, M., Effect of ferric oxyhydroxide grain coatings on the transport of bacteriophage PRD1 and *Cryptosporidium parvum* oocysts in saturated porous media. *Environ. Sci. Technol.* **2005**, 39, (17), 6412-6419.
52. Mohanram, A.; Ray, C.; Harvey, R. W.; Metge, D. W.; Ryan, J. N.; Chorover, J.; Eberl, D. D., Comparison of transport and attachment behaviors of *Cryptosporidium parvum* oocysts and oocyst-sized microspheres being advected through three mineralogically different granular porous media. *Water Res.* **2010**, 44, (18), 5334-5344.
53. Kim, S.-B.; Park, S.-J.; Lee, C.-G.; Kim, H.-C., Transport and retention of *Escherichia coli* in a mixture of quartz, Al-coated and Fe-coated sands. *Hydrological Processes* **2008**, 22, (18), 3856-3863.
54. Fisher, D. J., Charges on fungal spores. *Pestic. Sci.* **1973**, 4, (6), 845-860.
55. Fisher, D. J.; Richmond, D. V., The electrokinetic properties of some fungal spores. *J. Gen. Microbiol.* **1969**, 57, (1), 51-60.
56. Dorward, D. W.; Powell, M. J., Cytochemical Detection of Polysaccharides and the Ultrastructure of the Cell Coat of Zoospores of *Chytridiomyces aureus* and *Chytridiomyces hyalinus*. *Mycologia* **1983**, 75, (2), 209-220.
57. Gubler, F.; Hardham, A. R., Secretion of adhesive material during encystment of *Phytophthora cinnamomi* zoospores, characterized by immunogold labelling with monoclonal antibodies to components of peripheral vesicles. *J. Cell Sci.* **1988**, 90, (2), 225-235.
58. Sing, V. O.; Bartnicki-Garcia, S., Adhesion of *Phytophthora palmivora* zoospores: electron microscopy of cell attachment and cyst wall fibril formation. *J. Cell Sci.* **1975**, 18, (1), 123-132.
59. Robold, A. V.; Hardham, A. R., During attachment *Phytophthora* spores secrete proteins containing thrombospondin type 1 repeats. *Curr. Genet.* **2005**, 47, (5), 307-315.
60. Bradford, S. A.; Tadassa, Y. E.; Pachepsky, Y., Transport of *Giardia* and manure suspensions in saturated porous media. *J. Environ. Qual.* **2006**, 35, (3), 749-757.
61. Bradford, S. A.; Bettahar, M., Straining, attachment, and detachment of *Cryptosporidium* oocysts in saturated porous media. *J. Environ. Qual.* **2005**, 34, (2),

469-478.

62. Bradford, S. A.; Simunek, J.; Bettahar, M.; Van Genuchten, M. T.; Yates, S. R., Modeling colloid attachment, straining, and exclusion in saturated porous media. *Environ. Sci. Technol.* **2003**, *37*, (10), 2242-2250.
63. Bradford, S. A.; Simunek, J.; Bettahar, M.; van Genuchten, M. T.; Yates, S. R., Significance of straining in colloid deposition: Evidence and implications. *Water Resour. Res.* **2006**, *42*, (12), W12S15.
64. Torkzaban, S.; Bradford, S. A.; Walker, S. L., Resolving the coupled effects of hydrodynamics and DLVO forces on colloid attachment in porous media. *Langmuir* **2007**, *23*, (19), 9652-9660.
65. Bradford, S. A.; Torkzaban, S.; Walker, S. L., Coupling of physical and chemical mechanisms of colloid straining in saturated porous media. *Water Res.* **2007**, *41*, (13), 3012-3024.
66. Zevi, Y.; Dathe, A.; Gao, B.; Zhang, W.; Richards, B. K.; Steenhuis, T. S., Transport and retention of colloidal particles in partially saturated porous media: Effect of ionic strength. *Water Resour. Res.* **2009**, *45*, (12), W12403.
67. Zevi, Y.; Gao, B.; Zhang, W.; Morales, V. L.; Cakmak, M. E.; Medrano, E. A.; Sang, W.; Steenhuis, T. S., Colloid retention at the meniscus-wall contact line in an open microchannel. *Water Res.* **2012**, *46*, (2), 295-306.
68. Kim, H. N.; Walker, S. L.; Bradford, S. A., Coupled factors influencing the transport and retention of *Cryptosporidium parvum* oocysts in saturated porous media. *Water Res.* **2010**, *44*, (4), 1213-1223.
69. Kim, H. N.; Bradford, S. A.; Walker, S. L., *Escherichia coli* O157:H7 transport in saturated porous media: Role of solution chemistry and surface macromolecules. *Environ. Sci. Technol.* **2009**, *43*, (12), 4340-4347.
70. von Broembsen, S. L.; Deacon, J. W., Calcium Interference with Zoospore Biology and Infectivity of *Phytophthora parasitica* in Nutrient Irrigation Solutions. *Phytopathology* **1997**, *87*, (5), 522-528.
71. Granke, L. L.; Hausbeck, M. K., Effects of Temperature, Concentration, Age, and Algaecides on *Phytophthora capsici* Zoospore Infectivity. *Plant Dis.* **2010**, *94*, (1), 54-60.
72. Gregory, J., Approximate expressions for retarded van der waals interaction. *J. Colloid Interface Sci.* **1981**, *83*, (1), 138-145.
73. Hoek, E. M. V.; Agarwal, G. K., Extended DLVO interactions between spherical particles and rough surfaces. *J. Colloid Interface Sci.* **2006**, *298*, (1), 50-58.

74. Hogg, R.; Healy, T. W.; Fuerstenau, D. W., Mutual coagulation of colloidal dispersions. *Transactions of the Faraday Society* **1966**, 62, 1638-1651.
75. Ruckenstein, E.; Prieve, D. C., Adsorption and desorption of particles and their chromatographic separation. *AIChE J.* **1976**, 22, (2), 276-283.
76. Redman, J. A.; Walker, S. L.; Elimelech, M., Bacterial adhesion and transport in porous media: Role of the secondary energy minimum. *Environ. Sci. Technol.* **2004**, 38, (6), 1777-1785.
77. Kuznar, Z. A.; Elimelech, M., Adhesion kinetics of viable *Cryptosporidium parvum* oocysts to quartz surfaces. *Environ. Sci. Technol.* **2004**, 38, (24), 6839-6845.
78. van Oss, C. J., *Interfacial forces in aqueous media*. 2nd ed.; CRC Taylor & Francis: Boca Raton, FL, 2006.
79. Hahn, M. W.; O'Melia, C. R., Deposition and reentrainment of Brownian particles in porous media under unfavorable chemical conditions: Some concepts and applications. *Environ. Sci. Technol.* **2004**, 38, (1), 210-220.
80. Shen, C.; Li, B.; Huang, Y.; Jin, Y., Kinetics of coupled primary- and secondary-minimum deposition of colloids under unfavorable chemical conditions. *Environ. Sci. Technol.* **2007**, 41, (20), 6976-6982.
81. Tufenkji, N.; Elimelech, M., Correlation equation for predicting single-collector efficiency in physicochemical filtration in saturated porous media. *Environ. Sci. Technol.* **2004**, 38, (2), 529-536.

CHAPTER 3

FILTRATION OF PYTHIUM APHANIDERMATUM ZOOSPORES IN RECYCLED IRRIGATION WATER TO CONTROL POINSETTIA DISEASE IN GREENHOUSES

ABSTRACT

Pythium aphanidermatum incites crown and root rot and can be highly destructive to floriculture greenhouse crops especially when recirculating irrigation water systems are used. This study aimed to demonstrate a proof-of-concept using fast-flow filtration to control *Pythium* root rot of poinsettia grown with ebb-and-flow and flood-floor irrigation systems in commercial greenhouses. Two experiments were performed in a research greenhouse to investigate the effect of filter media type (i.e., sand and activated carbon), fungicide application (i.e., etridiazole) and pathogen inoculum source (i.e., infected plants vs infested irrigation water). The fast-flow sand filtration with low water pressure consistently removed *Pythium aphanidermatum* zoospores, and significant improvements in root rot severity, height, biomass, and horticultural rating were observed for the plants in the sand filter treatment, compared with the inoculated control plants. However, the activated carbon filter removed essential nutrients from the irrigation water, resulting in plant nutrient deficiency and consequently leaf chlorosis and reduced plant biomass, height, and horticultural rating. The application of etridiazole did not completely prevent root infection by *P. aphanidermatum*, but the plant biomass, height, and horticultural rating were not negatively affected by the pathogen. *P. aphanidermatum* spread from infected plants to healthy plants with the recycled irrigation water. Overall, the rapid sand filter has the potential to limit *P. aphanidermatum* spread and the associated root rot in

greenhouse floriculture crops.

3.1. INTRODUCTION

Floriculture crops in the U.S. have an estimated wholesale value of \$4.4 billion, and include a diverse assortment of bedding plants, potted flowers, and nursery crops. Poinsettias contributed a wholesale value of \$140 million in 2015 and are one of the top potted flowering plants in the U.S.^{1, 2} In the greenhouse production of floriculture crops, recirculating irrigation systems have been widely adopted to lower water usage, and conserve fertilizers that can otherwise be lost via discharge runoff.³⁻⁷ This is especially true for greenhouses with large water use, i.e., 0.5–1 million gallons per day.⁸ Irrigation systems with ebb-and-flow and flood-floor are also used to maximize production area and decrease labor costs.^{9, 10} In this type of irrigation system, irrigation water is pumped from a water reservoir to flood a floor at a specified water level for a desired duration, and then drained back (often by gravity flow) to the reservoir for recycling in the next irrigation event. Although recycling irrigation water offers many benefits to greenhouse growers, plant pathogens can also be disseminated in the recycled irrigation water.¹⁰ Thus, limiting pathogen transmission in the recirculating irrigation systems is critical to the floriculture industry.

Pythium spp. and other water molds can be highly destructive to floriculture crops, and spread readily in irrigation water.¹¹⁻¹⁵ *Pythium* root rot causes plant wilting and death and reduces horticultural quality.¹⁶ The pathogen can become established in a greenhouse through infested soil and dust¹⁷, seedlings, cuttings, or other plant material from propagation greenhouses,⁹ or infested surface water used for irrigation.³ Management of *Pythium* spp. is particularly challenging for potted plants; frequent

irrigation and high moisture levels are ideal for the reproduction and transmission of this pathogen.¹⁸ The high porosity of peat potting media may also facilitate the movement of zoospores, which are an important type of *Pythium* spp. inoculum.¹⁹ Thus, *Pythium* spp. could be rapidly disseminated in a greenhouse via ebb and flow flood floor production systems,²⁰ leading to crop damage and loss, thus requiring proactive management strategies.

Fungicide application is a common and important strategy to limit *Pythium* root rot in greenhouse production.²¹ Currently, the primary fungicides used for *Pythium* root rot control are etridiazole (Terrazole, OHP, Mainland, PA) and mefenoxam (Subdue Maxx, Syngenta Crop Protection, Greensborough, NC).^{21, 22} Etridiazole effectively reduced *Pythium* root rot in poinsettia and easter lily when applied as a soil drench.²²⁻²⁴ Also, etridiazole is one of the few commercial fungicides that is labeled for use in the ebb-and-flow and flood-floor irrigation systems.²⁵ Mefenoxam can also limit crop loss from *Pythium* root rot.²⁶ However, resistance to mefenoxam has developed in greenhouse populations of *Pythium* spp., partly due to repeated fungicide use.^{21, 26, 27} Failure to control *Pythium* diseases using mefenoxam has been reported in greenhouses,^{21, 28} and resistant isolates were detected in surface water used for irrigation.²⁹ Fungicide resistance has become a limiting factor for the control of *Pythium* crown and root rot; alternative strategies for pathogen control in irrigation water are needed.³⁰

Management of pathogens in recycled irrigation water is challenging. Ultra-violet radiation, heat treatment, chemical disinfection, ozonation, and filtration have been used to remove pathogens from irrigation water with varying degrees of success.^{10, 15} Many of these methods are cost-prohibitive to install and operate in commercial greenhouses. In

contrast, filtration is a low-cost method that could potentially disinfest irrigation water by the physical removal of pathogens using granular porous media (e.g., sand) or membrane filters.¹⁵ Membrane filtration can effectively remove zoospores if the membrane pore size is small enough to retain the motile zoospores that have a pleomorphic cell membrane.³¹ Membrane filters with pore sizes of 1 and 5 μm were able to effectively remove the *Pythium* zoospores from recirculating irrigation water in laboratory tests.³² However, it is unknown whether this could be transferable to greenhouse settings. Diplanetism (i.e., where a zoospore encysts and releases a smaller motile zoospore) could decrease the efficacy of membrane filters,³³ although the occurrence of diplanetism in commercial greenhouses is unknown. An additional challenge with membrane filters is frequent leakage, and membrane clogging and fouling^{10, 32}, resulting in increased maintenance cost and decreased performance over time.

In contrast, deep-bed filtration (e.g., sand filtration) is a cost-effective alternative in terms of construction, operation and maintenance. Slow filtration with granular materials has been studied as a means to remove *Pythium* spp. from the greenhouse irrigation water in the 1970s.³⁴ However, it isn't widely used in commercial U.S. greenhouses due to the slow water flow rate (i.e., 100–300 L/m²/h)¹⁰ that prohibits the movement of a large volume of water to multiple greenhouse ranges in an acceptable time period.¹⁵ Previous studies focused on the ability of slow sand filtration in limiting plant pathogens in irrigation water.^{10, 15, 35} The effectiveness of fast-flow filtration on pathogen removal from irrigation water has not been well investigated. Additional data could help to determine whether this technique could be adopted to manage irrigation water in greenhouses.

The objective of this study was to investigate the ability of fast-flow filtration to limit *Pythium* diseases for potted poinsettias in greenhouses with ebb-and-flow and flood-floor irrigation systems. Six small-scale ebb-and-flow recirculating irrigation systems were constructed to simultaneously test the effect of filter media type (i.e., sand and activated carbon), fungicide application (i.e., etridiazole), and pathogen transmission mode (i.e., infected plants vs infested water). Poinsettia was used as a model crop because of its popularity as a potted flower and the prevalence of *Pythium* outbreaks in its production. This study was intended to show a proof-of-concept to use the fast-flow filtration system in removing plant pathogens from recirculating irrigation water.

3.2. MATERIALS AND METHODS

3.2.1. Irrigation and Filtration Systems.

Six small-scale irrigation systems were constructed to simulate the ebb-and-flow and flood-floor systems in greenhouse settings, consisting of an ebb-and-flow bench, an optional pre-filter tank, an optional filter unit, and a holding tank (Figure 3.S1). Only one holding tank was included in some of filter-free treatments, including the non-inoculated and inoculated control treatment in the first experiment, and in the inoculated control and “diseased plant” treatments in the second experiment. Otherwise, the pre-filter tank and the holding tank were directly connected for the non-filtration treatments. The filter unit was designed as shown in Figure 3.S2, and was packed with either sand (99.69% silica, Granusil®) or activated carbon (AC) (Filtrisorb 300, CalgonCarbon, USA). Particle size distribution of the sand was 5.1% of 297-420 µm, 57.2% of 420-595 µm, 36.1% of 595-841 µm, and 1.2 % of ≥ 841 µm. The effective size of AC particles was 0.8-1.0 mm. Detailed description on the irrigation systems and filter units is provided in Supporting

Information S1. The irrigation systems allowed for automatic irrigation of potted plants placed in the bench via flooding according to a pre-designated schedule. These irrigation systems were subsequently used in two greenhouse experiments.

3.2.2. Pathogen Culture and Inoculum.

Pythium aphanidermatum is one of the most prevalent *Pythium* species in greenhouses, and is more aggressive on poinsettia than the another prevalent species *P. irregulare*.²⁷ *P. aphanidermatum* isolates 106 and 319 were previously characterized for sensitivity to etridiazole,² and were selected from the culture collection of Dr. M.K. Hausbeck at Michigan State University (MSU) and maintained on corn meal agar (CMA: 17 g/L corn meal agar). Prior to the study, the isolates were inoculated on poinsettia stems and subsequently re-isolated from the diseased stems to ensure virulence.³⁶ Zoospores of *P. aphanidermatum* were produced according to a previously established method as described in SI.³⁷ The concentration of initially produced zoospores was $1.7 \pm 0.9 \times 10^4$ zoospores/mL. The prepared suspension of the motile biflagellate zoospores were equally split into five 500-mL capped bottles, and hand-shaken vigorously for 90 seconds to induce zoospore encystment. These suspensions of encysted zoospores were then used later as the inoculum in irrigation water and for the poinsettias.

3.2.3. Plant and Irrigation Water.

One-month old cuttings of poinsettia (*Euphorbia pulcherrima*) Early Prestige Red were obtained from a local commercial greenhouse. The cuttings were transplanted into plastic nursery pots of 15 cm in diameter and 10.5 cm in height packed with a peat potting mixture (Suremix, SunGro, Galesburg, MI). Fifteen plants were placed on each of the six ebb-and-flow benches to ensure a sufficient number of replicates. In the second

experiment, the poinsettia cuttings were propagated from mature plants following a commercial propagation recommendation³⁸ and planted into the nursery pots as described above. The poinsettia plants were approximately 6-week old at the start of each experiment. A 20-5-19 (N-P-K) water-soluble fertilizer (JR Peters Inc, PA, USA) was added to the irrigation water, and the initial nutrient concentration was 125 mg/L based on nitrogen. Ground water (pH 7.85 ± 0.22) was used as the source of irrigation water. The pre-filter tank (or the holding tank in some filter-free treatments) in the irrigation systems was filled with 120 L of the fertilized irrigation water. Plants were placed on the benchtops with irrigation in operation for 2-days prior to inoculation to acclimate to the soluble salts in solution. The suspension of encysted zoospores (490 mL of 1.7×10^4 zoospores/mL) was added to the pre-filter or holding tank and thoroughly agitated with a wooden dowel. The resultant concentration of the encysted zoospores was 68 ± 36 zoospores/mL. During the greenhouse experiments, the irrigation water was passed through the filter unit and then stored in the holding tank immediately prior to an irrigation event. Two additional inoculations were made two and four weeks after the initiation of the experiment to increase disease pressure. To maintain a sufficient water amount during each experiment, the fertilized irrigation water was added when the water volume decreased to about 70–80% of the initial volume.

3.2.4. Greenhouse Experiments.

Two greenhouse experiments were conducted in the temperature-controlled greenhouse under six experimental treatments, including: 1) a non-inoculated control treatment without filtration and pathogen (–Control); 2) inoculated control treatment without filtration or fungicide (+Control); 3) inoculated treatment with the sand filter; 4)

inoculated treatment with the AC filter; 5) inoculated treatment with the fungicide etridiazole (Terrazole 35WP) application; and 6) ‘diseased plant’ treatment. In treatment 5, the etridiazole was applied at the labeled rate (250 mg/L) into the irrigation water in the holding tank before the first inoculation to assess the effectiveness of the fungicide treatment, in which no filter unit was used. In the “diseased plant” treatment (Treatment 6), 3 out of the 15 plants were directly inoculated with the *Pythium* zoospores, and then placed randomly in the first experiment and at the back location near the drainage hole of the bench in the second experiment, as shown in Figure 3.S3. In the “diseased plant” treatment, *Pythium* zoospores were not introduced into the irrigation water, but were used to inoculate the poinsettia plants. Briefly, 50 mL of the encysted zoospore suspension was added to a 4-cm deep depression in the potting mix 2-cm from the stem of the healthy plant. These pots were removed from the bench during inoculation to avoid contamination of the bench, and then placed on the bench after the inoculation. This treatment was designed to assess whether the pathogen could spread among the plants if not directly introduced into the irrigation water. The poinsettia plants were irrigated twice a day at 0900 and 1500 hr. The first experiment was initiated on October 29, 2014 and concluded on January 6, 2015 (69 days in duration). The second experiment was initiated on April 2, 2015 and concluded on June 19, 2015 (78 days in duration). The initiation day was considered to be that of the first inoculation. Due to external streetlights providing light contamination, the plants in the first experiment experienced an interruption of the dark period that is required to initiate the flowering. Thus, in the second experiment, the plants were covered with a thick black cloth at night to initiate flowering. The water pressure inside of the filter unit was monitored in real time, along with the water

temperature in the holding tank, and air temperature and relative humidity in the greenhouse, as detailed in SI. The irrigation water was sampled from the holding tanks at the beginning, middle, and end of each experiment to determine the pH, electrical conductivity (EC), and nutrient concentrations. After the pH and EC measurements, the water samples were filtered through the 0.45- μ m and stored in a -20°C freezer for nutrients analyses later by the MSU Soil and Plant Nutrient Laboratory.

3.2.5. Plant Assessments.

To evaluate the performance of the filtration systems in controlling *Pythium* root rot outbreaks, the poinsettia plants were evaluated at the end of the experiment for foliar and root biomass, root rot severity, and horticultural quality. The roots were evaluated for root necrosis using a scale adapted from Boehm and Hoitink,³⁹ where: 1 = no symptoms; 2 = mild root rot, $<1/3$ affected; 3 = intermediate root rot, $1/3$ to $2/3$ affected; 4 = severe root rot, $>2/3$ roots affected; 5 = severe root and crown rot; and 6 = dead plant. This rating was made without removing the potting mix from the roots. In the second experiment, the plants were rated for their horticultural quality³⁸ based on the appearance (e.g., color, height and bract area) on the scale from 1 (high aesthetic quality) to 5 (no aesthetic quality) (Figures S4). At harvest, the roots were carefully washed and the fresh biomass of the poinsettia shoots and roots were measured. The shoot and root samples were then oven-dried at 60°C for 3 days and measured for their dry biomass. Isolation of *P. aphanidermatum* from the roots of each plant was attempted to determine the root infection ratio (IR) as described in SI.

Chlorophyll a, and b concentrations in the poinsettia leaves were measured using the colorimetric method,⁴⁰ as described in SI. To analyze the macro- and micro- nutrients

in the poinsettia leaves, the stems were removed and the remaining leaf tissues were ground before being analyzed at the MSU Soil and Plant Nutrient Laboratory following the standard methods.

3.2.6. Statistical Analysis.

Statistical analyses of the experimental data were performed with R software using the “LSD” R package for parametric tests and the “coin” R package for a nonparametric test such as the rating data (i.e., the ratings of root rot severity and horticultural quality). Treatments were compared by one-way analysis of variance ($p \leq 0.05$). When a significant F value was determined, means were separated by the LSD’s multiple comparison test. Also, the student’s t -test was used to compare paired samples. Data of pre-inoculated plants in the ‘diseased plant’ treatment were not included in the statistical analyses. To compare nonparametric data, such as root rot severity and horticulture rating, the Kruskal-Wallis test were used with the “coin” R package. Its statistical significance p -value was adjusted ($p \leq 0.034$) by Bonferroni correction to reduce the risk of committing Type I errors for multiple comparison.

3.3. RESULTS

3.3.1. Irrigation and Filtration Systems.

Six small-scale ebb-and-flow irrigation systems (Figure 3.S1) and filtration units (Figure 3.S2) were used to evaluate the effectiveness of filtration and fungicide against *P. aphanidermatum* and to demonstrate the pathogen dissemination among plants. The air temperature and relative humidity were 26.8 ± 2.9 °C and 30 ± 7.4 % in the first experiment, and 26.1 ± 3.3 °C and 36 ± 16.7 % in the second experiment, respectively

(Figure 3.S5). The water flow velocities through the AC and sand filters were 19.6 ± 0.5 and 10.5 ± 2.2 cm/min in the first experiment, and 18.6 ± 1.3 and 8.6 ± 1.0 cm/min in the second experiment, respectively. Consequently, the water residence time in the AC and sand filters was 2.5 ± 0.1 and 4.9 ± 0.9 minutes in the first experiment, and 2.6 ± 0.3 and 6.3 ± 0.4 minutes in the second experiment, respectively. The water flow velocity and residence time were calculated based on three-day averages at the beginning of each experiment ($n = 6$). Thus, the two experiments had consistent water velocities through the filters. Operating water pressure of the AC and sand filters were maintained at 6.9 ± 1.4 kPa (i.e., 1.0 ± 0.2 psi) and 5.73 ± 1.00 kPa (i.e., 0.83 ± 0.15 psi), respectively (Figure 3.S6). Because filtration systems with a water velocity of 8.3–25 cm/min are classified as rapid sand filtration,^{41–43} relative to slow sand filtration (0.17–0.5 cm/min), these filtration systems are classified as fast-flow rate (8.6–19.6 cm/min) and low pressure. It was noted that after about one month from the start of the second experiment, a significant water flow reduction was observed, likely due to clogging by debris and biofilm.⁴⁴ Flow reduction due to clogging has also been observed with slow sand filters,³¹ and can often be alleviated by backwashing.³² To maintain the proper water flow rate, backwashing was conducted once a week after one month and then every other day during the last three weeks in the second experiment. No significant reduction of water flow rate was found in the first experiment so, backwashing was only performed a couple of times during the final month of the experiment.

The pH and EC of the irrigation water were 7.8 ± 0.4 and 1.6 ± 0.3 mS, respectively, during the experimental period (Figure 3.S7 and S8); water temperature was in the range typical for a greenhouse (Figure 3.S9). The concentrations of macronutrients

(i.e., NO_3^- , P, K, Ca, Mg, and Na) in the irrigation water are shown in Table 3.S1. The nutrient levels (i.e., NO_3^- , P, K, Ca, and Mg) in the AC filter treatment were generally lower than those of other treatments, although the difference was less in the second experiment. Thus, it appeared that the AC removed these nutrients from the irrigation water⁴⁵⁻⁴⁹ and the final nutrient levels in the irrigation water were dependent on the nutrient absorption by the poinsettia roots and the nutrient removal by the AC filter. It appeared that the severe root rot in the AC filter treatment during the second experiment (as shown later) may have caused insufficient nutrient absorption and subsequently higher nutrient levels than those in the first experiment. Moreover, the micronutrients (i.e., Fe, Cu, and Zn) were completely removed from the recycled irrigation water in the AC filter treatment (Figure 3.S10). Thus, in both experiments there might be nutrient deficiency for the plants in the AC filter treatment.

3.3.2. Horticultural rating.

The aesthetic quality of the poinsettias was assessed in the horticultural rating scale from 1 to 5 at the end of the second experiment (Table 3.1 and Figure 3.1). The plants in the first experiments did not properly develop the red color leaves because of the interruption of the dark period by light contamination (Figure 3.S11), and thus could not be evaluated for aesthetic quality. The inoculated control plants had an inferior appearance with a horticultural rating of 3.0, and were not marketable. The plants subjected to the AC filter treatment had the poorest aesthetic quality with a horticultural rating of 4.7, whereas the plants in the remainder of the treatments received similar horticultural ratings (1.4–2.3, Table 3.1).

3.3.3. Plant Assessment.

The inoculated control plants displayed severe root rot and stunting in both experiments (Table 3.1, Figure 3.1, 2, S11, and S12). Root rot severity was visually determined by assessing the percentage of root necrosis using a scale from 1 to 5 (Figure 3.S12 and Table 3.1). In the first experiment, the level of root necrosis in the inoculated control plants (3.8 ± 1.0) was significantly ($p < 0.0034$) more than that of the non-inoculated control (1.7 ± 1.0), sand filter (1.7 ± 0.9), AC filter (1.4 ± 0.5), and “diseased plant” (1.8 ± 1.1) treatments. Root necrosis in the etridiazole treatment (2.5 ± 1.2) was the second highest. In the second experiment, the levels of root necrosis in the inoculated control plants (3.2 ± 0.9), AC filter (3.2 ± 0.6), and etridiazole (3.0 ± 0.7) treatments were significantly higher ($p < 0.0034$) than those of the non-inoculated control (1.1 ± 0.4), sand filter (1.2 ± 0.4) and ‘diseased plant’ (1.5 ± 1.2) treatments (Table 3.1). In particular, the roots of plants in the AC filter treatment showed significant root necrosis (Figure 3.S12B) although *P. aphanidermatum* was not isolated from the roots. Presence of root symptoms did not always correspond with isolation of *P. aphanidermatum*, perhaps due to isolation inefficiency. In the first experiment, *P. aphanidermatum* was isolated from the roots of 93, 80, and 50% of the plants for the inoculated control, etridiazole, and “diseased plant” treatments, respectively, and the pathogen was not isolated from the roots of the non-inoculated control, AC, and sand filter treatments (Figure 3.2). The presence of *P. aphanidermatum* in the “disease plant” treatment did not result in significant difference in the root necrosis compared with that of the non-inoculated control treatment. It is likely due to the masking effect from other healthy plant roots (Figure 3.S3). In the second experiment, *P. aphanidermatum* was isolated only from the

roots in the inoculated control (93% incidence). Significant root necrosis was observed for some plants in the AC filter, “diseased plant”, and etridiazole treatments. It is possible that rinsing the roots with water and surface-sterilizing them with 70% ethanol prior to isolation prevented pathogen recovery. The root necrosis in the AC filter and etridiazole treatments might result from nutrient deficiency and phytotoxicity respectively, whereas it remained unexplained for the “diseased plant” treatment. It is important to note that plants in the sand filter treatment consistently showed healthy roots similar to those of the non-inoculated control, demonstrating the effectiveness of rapid sand filtration in limiting *Pythium* infection.

Plant height and biomass were assessed to determine whether filtration effectively maintained plant quality. In the first experiment, the sand filter, etridiazole, “diseased plant”, and non-inoculated control treatments had significantly higher plant height, foliar fresh weight, and root dry weight than those of the inoculated control plants ($p < 0.05$, Figure 3.3). However, plants in the AC filter treatment had significantly lower fresh foliar weight and root dry weight than those of the non-inoculated control ($p < 0.05$, Figure 3.3). In the second experiment, the sand filter, etridiazole, “diseased plant” and non-inoculated control treatments had significantly higher plant height, and foliar fresh and dry weight than those of the inoculated control and AC filter treatment ($p < 0.05$) (Figure 3.S12).

Chlorosis of young leaves was observed for plants in the AC filter treatment in both experiments (Figure 3.S14). Chlorophyll a and b analysis for the medium-sized leaves after harvest were significantly lower in the AC filter treatment than those of other treatments (Table 3.2). The leaves had high levels of S and Ca and low levels of B, Zn,

Fe and Cu in the AC filter treatment, relative to the non-inoculated control. There was no significant difference in the concentrations of N, P, K, Mg, Na, Mn, and Al in the leaves from the AC filter treatment and the non-inoculated control treatments (Table 3.S2).

Table 3.1. Root rot severity and horticultural rating in the first and second experiments.

Exp. #	Parameter	–Control	+Control	AC filter	Sand filter	Diseased plant	Etridiazole
1st expt.	Root rot severity	1.7 ± 1.0 a*	3.8 ± 1.0 b	1.4 ± 0.5 a	1.7 ± 0.9 a	1.8 ± 1.1 a	2.5 ± 1.2 ab
2nd expt.	Root rot severity	1.1 ± 0.4 a	3.2 ± 0.9 b	3.2 ± 0.6 b	1.2 ± 0.4 a	1.5 ± 1.2 a	3.0 ± 0.7 b
	Horticultural rating	1.4 ± 0.6 a	3.0 ± 0.8 b	4.7 ± 0.5 c	1.7 ± 0.5 a	2.3 ± 0.8 ab	1.9 ± 0.6 a

*Kruskal-Wallis with Bonferroni corrected, $P < 0.0034$

Table 3.2. Chlorophyll a and b in the first and second experiments.

		–Control	+Control	AC filter	Sand filter	Diseased plant	Etridiazole
1st expt.	Chlorophyll a	42.71 bc*	49.09 a	16.48 d	39.16 c	47.93 a	46.12 ab
	Chlorophyll b	76.28 bc	89.41 a	25.98 d	68.07 c	80.63 b	76.92 b
2nd expt.	Chlorophyll a	16.2 ab	13.2 bc	4.0 d	13.0 c	15.1 abc	17.3 a
	Chlorophyll b	110.4 ab	91.1 bc	25.4 f	88.6 c	101.9 abc	117.3 a

*LSD test, $P < 0.05$



Figure 3.1. Poinsettias at the end of the second experiment (i.e., 78 days after inoculation).

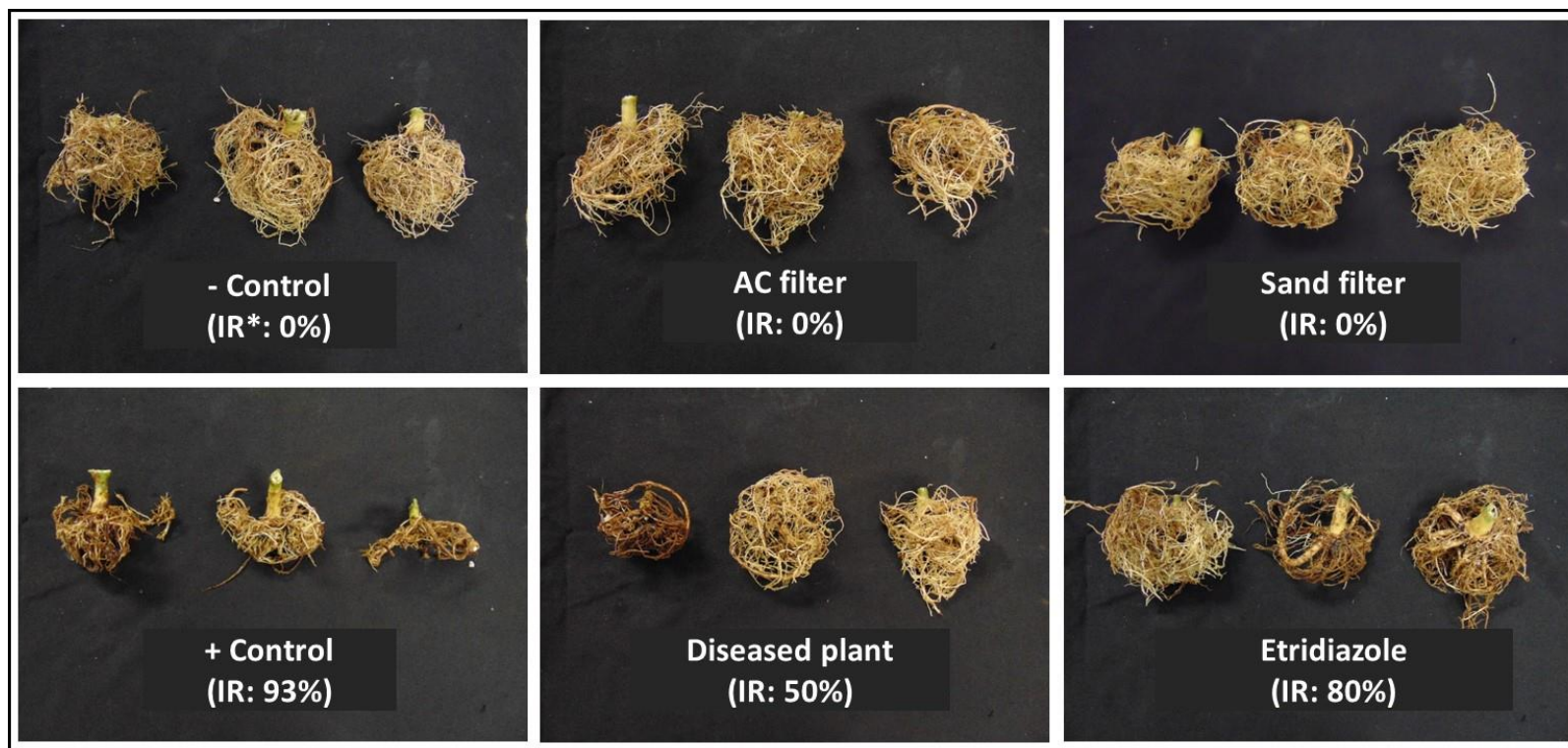


Figure 3.2. Roots of the poinsettias at the end of the first experiment (i.e., 69 days after inoculation). IR: Infection ratio of Pythium in roots.

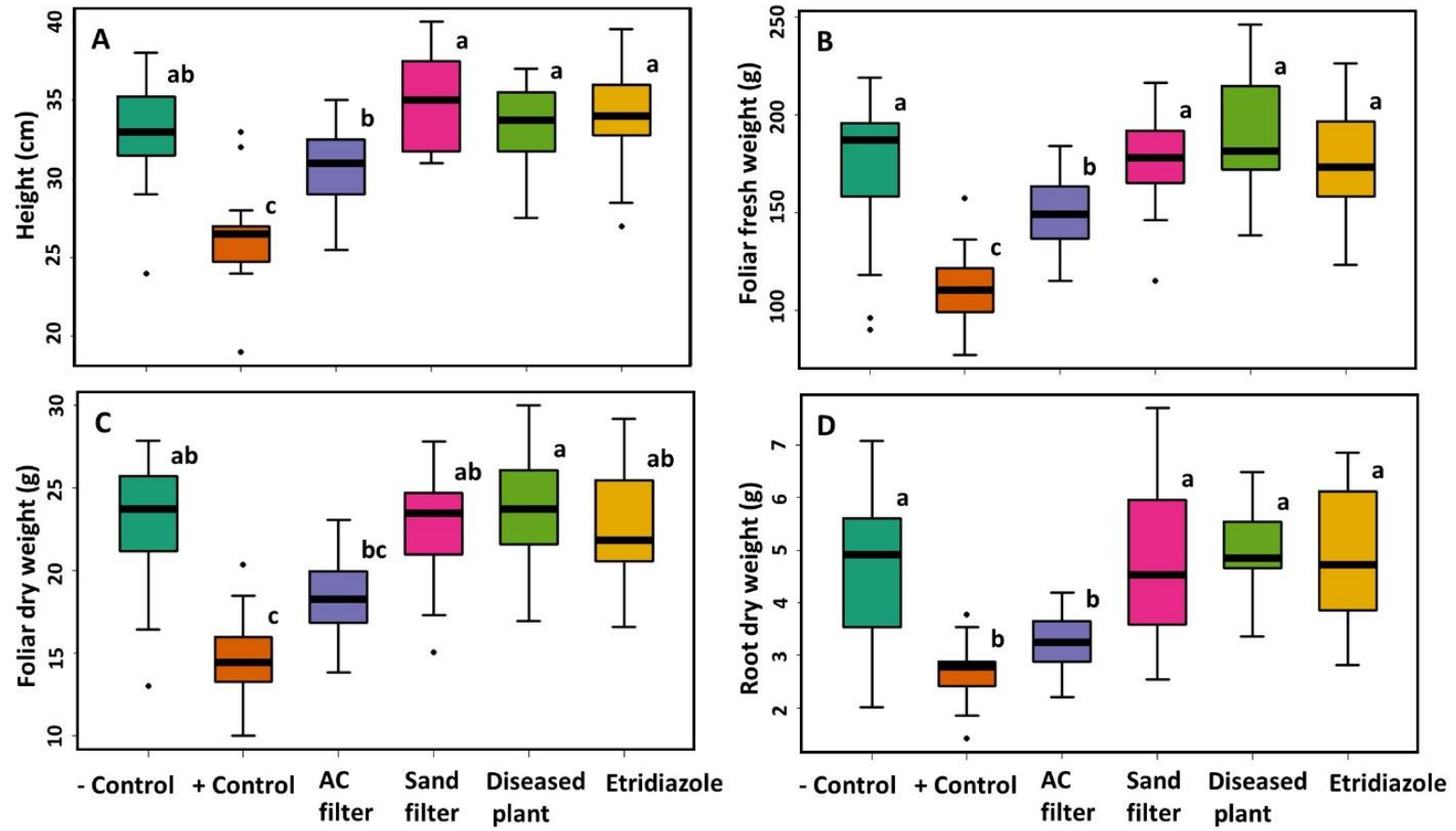


Figure 3.3. Poinsettia height (A), foliar fresh biomass (B), foliar dry biomass (C), and root dry biomass (D) in the first experiment (LSD test, $P < 0.05$).

3.4. DISCUSSION

3.4.1. Filtration Performance.

Our results demonstrated that the poinsettia plants under the fast-flow sand filtration were consistently similar to the non-inoculated control, regarding the presence of *P. aphanidermatum*, root necrosis, plant height and biomass, and horticultural quality. Thus, the rapid sand filtration effectively limited *P. aphanidermatum*, likely by removing *P. aphanidermatum* zoospores from the irrigation water. Many laboratory and greenhouse experiments have consistently shown that sand filters can effectively remove phythiaceus zoospores from water.^{35, 50-55} Similar results have also been found with the slow sand filtration under experimental conditions.^{35, 53, 54, 56} The slow sand filtration was initially developed for wastewater treatment through biological processes; a biological layer termed “Schmutzdecke” is the most critical factor for purification.⁴² However, others suggested that the main mechanisms to remove phythiaceus zoospores primarily rely on physicochemical factors including surface attachment, pore straining, and adhesive interactions of the zoospores in the porous media.^{50, 53, 54, 56} Thus, the physiochemically-controlled fast-flow filtration can be a viable alternative for treating recycled irrigation water in the greenhouse. Slow sand filtration is often not suitable for greenhouse production due to its low water flow rate and the large footprint of the filtration system.⁵⁷ In this study, the flow rate of the rapid sand filter was about 40–50 times of that in typical slow sand filters, and would thus would meet the water demand in commercial greenhouses with a small footprint.

Nonetheless, a reduction was observed in the water flow rate in the sand filter treatment over time, potentially due to clogging of the sand filter by debris or biofilm.⁴⁴

The clogging can usually be easily remediated by backwash that is often performed in typical filtration operations.³² Recently, Kim et al.⁵⁸ reported that a pungent oil of fresh ginger (6 gingerol) reduced *Pseudomonas aeruginosa* biofilm formation up to 53% by inhibiting quorum sensing-regulated virulence behaviors. In addition, several quorum sensing inhibitors including RNAlII-inhibiting peptide, usnic acid, and a natural secondary metabolite of lichen could also inhibit biofilm formation.⁵⁹ However, no study has been conducted on whether the quorum sensing inhibitors can maintain the water flow rate in a sand filter. Thus, we used the backwash to maintain the water flow rate in this study. Since performing the backwash sustained the desired water flow rate in our sand filters, using rapid sand filtration may be an option to limit *Pythium* spp. in recycled irrigation water in commercial greenhouses due to its low cost to install, maintain, and operate.¹⁰

The AC filter effectively removed *P. aphanidermatum* zoospores, and no flow reduction was observed during the two experiments. However, the biomass and height of the plants under the AC filter treatment were, in general, significantly reduced compared to the non-inoculated and sand filter treatments (Figure 3.3 and S13). Because *P. aphanidermatum* was not detected in the relatively healthy-appearing roots in the AC filter treatment of the first experiment, the leaf chlorosis and stunted growth may have resulted from abiotic factors. As the AC removed essential micronutrients (e.g., Fe, Cu, Mn, and Zn) from the irrigation water (Figure 3.S10), and the concentrations of Fe, Cu, and B in the plant leaves (Table 3.S2) were in the deficient range,^{60, 61} the leaf chlorosis might be due to micronutrient deficiency. The light green coloration of young leaves and chlorosis indicate Fe deficiency.^{62, 63} Similarly, the kiwifruit showed the Fe deficient

symptom when the soil was amended with a wood-based biochar.⁶⁴ Biochar produced by pyrolysis characterized by carbon-rich, large surface area, high porosity, and lot of functional groups similar to that of AC.^{65, 66} The removal of nutrients by the AC may prevent its adoption for water treatments in commercial greenhouses.⁶⁷ Despite its effectiveness in removing the plant pathogen, the AC filters may not be ideal for the commercial poinsettia production due to poor plant quality (Table 3.1 and Figure 3.1)

3.4.2. Fungicide application.

In the etridiazole treatment across both experiments, the poinsettias had similar biomass and height to those in the non-inoculated control. However, root infection was not prevented by the fungicide application. *P. aphanidermatum* was isolated from roots exhibiting an intermediate level of root rot severity (Table 3.1 and Figure 3.2) in the first experiment. In the second experiment, the plant roots also displayed an intermediate level of root rot severity, but no *P. aphanidermatum* was isolated. When etridiazole was incorporated into the growing medium, necrosis was reduced at the stem base of cucumbers, but did not decrease recovery of *P. aphanidermatum* from the roots in an ebb-and-flood floor system.⁶ In a hydroponic system, etridiazole applied in the recirculating irrigation water reduced the root rot of *Hedera* spp., but not as effectively as the fungicide mefenoxam.⁶⁸ The presence of *P. aphanidermatum* in the poinsettia roots in the first experiment suggested that the rate of etridiazole in the irrigation water may not be adequate in controlling infection. Etridiazole did not cause 100% mortality to the zoospores of sensitive isolates of *P. aphanidermatum in vitro*.² Additionally, the application of sub-lethal doses of fungicides may increase the resistance of *Pythium* spp. to fungicides and exacerbate disease symptoms.⁶⁹ Plants may not be adequately

protected if the fungicide concentration is below the threshold necessary to prevent disease. Moreover, etridiazole may cause phytotoxicity in the poinsettia plants. Severe root rot occurred with the etridiazole treated water in the absence of *P. aphanidermatum* in the second experiment, suggesting that the recommended dosage of this fungicide may cause phytotoxicity in the roots or induce root rot symptoms.⁷⁰⁻⁷²

3.4.3. Pathogen inter-plant transmission.

To demonstrate pathogen transmission from plant to plant, a study was designed that randomly placed three inoculated poinsettia plants among 12 healthy plants. In the first experiment, *P. aphanidermatum* from inoculated plants was transferred to healthy plants. These infected plants did not show reduced biomass, but exhibited root rot symptoms by the end of experiment (Figure 3.S3A). *Pythium* transmission from inoculated to healthy plants was less efficient than infesting the irrigation water.¹⁴ The pathogen did not spread from inoculated to healthy plants when inoculated plants were placed at the back as shown in Figure 3.S3B. It is possible that the released zoospores from the infected roots could be drained away with the irrigation water, and then become attached to the surfaces of pipes and walls during the encystment.⁷³ It is unlikely that the zoospores would be transported against the flow direction as the motile zoospores can only move up to a few millimeters to centimeters by active swimming.⁷⁴⁻⁷⁶ Thus, the zoospores were possibly transported by irrigation water to infect the plants in the first experiment, as the infected plants were located along the flow direction in the middle of the bench (Figure 3.S3A).

3.5. IMPLICATIONS

In our study, *P. aphanidermatum* was effectively removed by the sand and AC filters during fast-flow filtration with low water pressure. The rapid sand filter maintained poinsettia quality compared to the non-inoculated control. The AC filter can also remove the essential nutrients from the irrigation water, and cause Fe deficiency symptoms, thus the use of an AC filter in the recirculating irrigation systems may not be preferred, unless the nutrients can be applied separately. The application of etridiazole did not completely prevent *Pythium* infection (e.g., root rots), but plant quality in terms of biomass, height and horticultural quality was not compromised. Thus, fungicides may still be needed to control the *Pythium* outbreak. When the poinsettia plants were randomly infected by *P. aphanidermatum*, the pathogen spread among plants in the absence of any treatment, suggesting the need of proactive measures to control the pathogen transmission either by fungicide application or filtration.

In summary, our proof-of-concept study suggests that filtration of irrigation water can effectively reduce crop disease outbreaks in greenhouses with ebb-and-flow and flood-floor production systems. This could decrease the use of fungicides and promote crop and environmental health. Future work should focus on assessing the longevity of the system performance by optimizing filter media and operation parameters. For instance, the filter design can be improved by incorporating anti-clogging mechanisms such as deeper coarse layer at the inlet, or removable screening for dislodging accumulated debris. Sand grain size could also be optimized to improve water flow, while maintaining the zoospore removal efficiency. When using the AC filter, the nutrients should be applied via ways other than irrigation water. Finally, the irrigation

frequency and duration, and backwashing scheduling may be optimized to ensure the continuous system performance.

3.6. ACKNOWLEDGMENTS

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APPENDIX

APPENDIX

SUPPORTING INFORMATION

S1. Supplemental Materials and Methods

S1.1 Construction of Ebb-and-Flow Irrigation Systems

To test the effectiveness of filtration units in controlling disease outbreaks in greenhouse-grown poinsettias, six self-contained ebb-and-flow irrigation systems (including optional filtration units) were constructed (Figure 3.S1**Error! Reference source not found.**). A typical irrigation system consisted of an 2.4 m × 1.2 m black plastic ebb-and-flow bench (Hummert, St. Louis), an optional filtration unit, two 130-L holding tanks, two 12V-centrifugal water pumps, two check valves, two auto valves, two water-level sensors, and a timer. The irrigation water was withdrew from the pre-filter tank by one water pump, passed through a check valve, the filter unit, an auto valve (i.e., 1/2-inch motorized ball valve, Model number: MV-2-20-12V-R01-1, Misol, China), and then stored in the holding tank until a pre-scheduled irrigation time controlled by a timer. At the time of irrigation, the irrigation water in the holding tank was pumped into the ebb-and-flow bench via a check valve until reaching a desired watering height (i.e., 3–4 cm or 10 mins of pumping time). The two check valves were installed to prevent the backflow. One check valve was next to the pump connected to the pre-filter tank, and the other next to the pump connected to the holding tank (Figure 3.S1). The irrigation water in the bench was kept for a desired irrigation period before being drained back to the pre-filter tank by opening an auto valve (i.e., the 3/4-inch motorized ball valve). Two magnetic float water-level sensors (Model number: a11062100ux0008, Uxcell, VA, USA) were installed in the pre-filter tank and holding tank, respectively. The water-level

sensor in the pre-filter tank detects the irrigation water drained from the bench, and then the water pump is automatically turned on to deliver the water to the inlet of the filter unit. The second water-level sensor turns off the water pump connected to the holding tank, if the water level reaches the minimum level so as to prevent air entry into the pump.

The filter unit design is described in detail next. Activated carbon (AC) and sand were used as filter media. Operating water pressure of the AC and sand filters was maintained at 6.9 ± 1.4 kPa (i.e., 1.0 ± 0.2 psi) and 5.73 ± 1.00 kPa (i.e., 0.83 ± 0.15 psi), respectively. The water pressure was measured by a pressure transducer with a range of 0–15 psi (Model: MK-15, China) at the top of the filters and then recorded in a data-logger (Model: MCR-4V; TandD, Japan). All of the irrigation systems were sterilized before each experiment. Specifically, a solution of > 30% of household bleach (i.e., 6.15% NaClO solution) was applied in the benches and tanks using sprayers, and any adhering grime or algae was removed using scrub brushes. Then, 10 L of a 5% bleach solution was added to the pre-filter tank and allowed to recirculate a couple of times in the absence of a filter unit. After the cleaning, the systems were thoroughly rinsed several times with tap water and air-dried for several days.

S1.2. Filter Unit Design

Low-pressure sand and AC filters were constructed for the greenhouse experiments (Figure 3.S2). Each filter unit was made with a PVC pipe of 50 cm in length and 15.2 cm (6 inches) in diameter. The bottom of each filter column was sealed with an end cap fitting and the top of each filter was assembled with a coupling, an adapter fitting, and a plug fitting in order. Two types of filter media, i.e., sand (99.69% silica,

Granusil[®]) and AC (Filtrisorb 300, CalgonCarbon, USA), were used. The particle size distribution of the sand was 5.1% of 297-420 μm , 57.2% of 420-595 μm , 36.1% of 595-841 μm , and 1.2 % of $\geq 841 \mu\text{m}$. The effective size of AC particles was 0.8-1.0 mm. A 3-cm layer of coarser sand (500–841 μm) was placed at the bottommost and upmost in the sand filter to filter out large debris and thus minimize the clogging, but only at the bottom of the AC filter. The total depth of filter media was 50 cm including the coarser sand layers. All of filter media were used directly without washing. To support the filter media and allow for the free drainage of the filtered water, two screens with different opening sizes (i.e., 12.7 mm $\times$ 12.7 mm and 6.35 mm $\times$ 6.35 mm) were prepared and bent to be fixed onto about the 2-cm length of the 15.2-cm PVC pipe using 12 screws and then mounted inside the end of the bottom cap. A stainless screen with 100 $\times$ 100 μm opening size was placed on the screens. The top of the filter media was also covered with the 100 $\times$ 100 μm and 12.7 $\times$ 12.7 mm screens in the similar manner as that at the bottom part to filter large debris and allow for an even distribution of water flow (Figure 3.S2). The bottom end cap was drilled and fitted with a 1/2-inch polypropylene bulkhead tank fitting (TF050, Banjo, USA) to connect the outlet pipe along with a union fitting (Mueller/B &K, USA) and a 1/2-inch motorized ball valve (Misol, China). The motorized ball valve was open during the operation of the pump connected to the pre-filter tank. The pressure sensors were installed at the inlet of each filter. All of the components were assembled and sealed to make watertight columns.

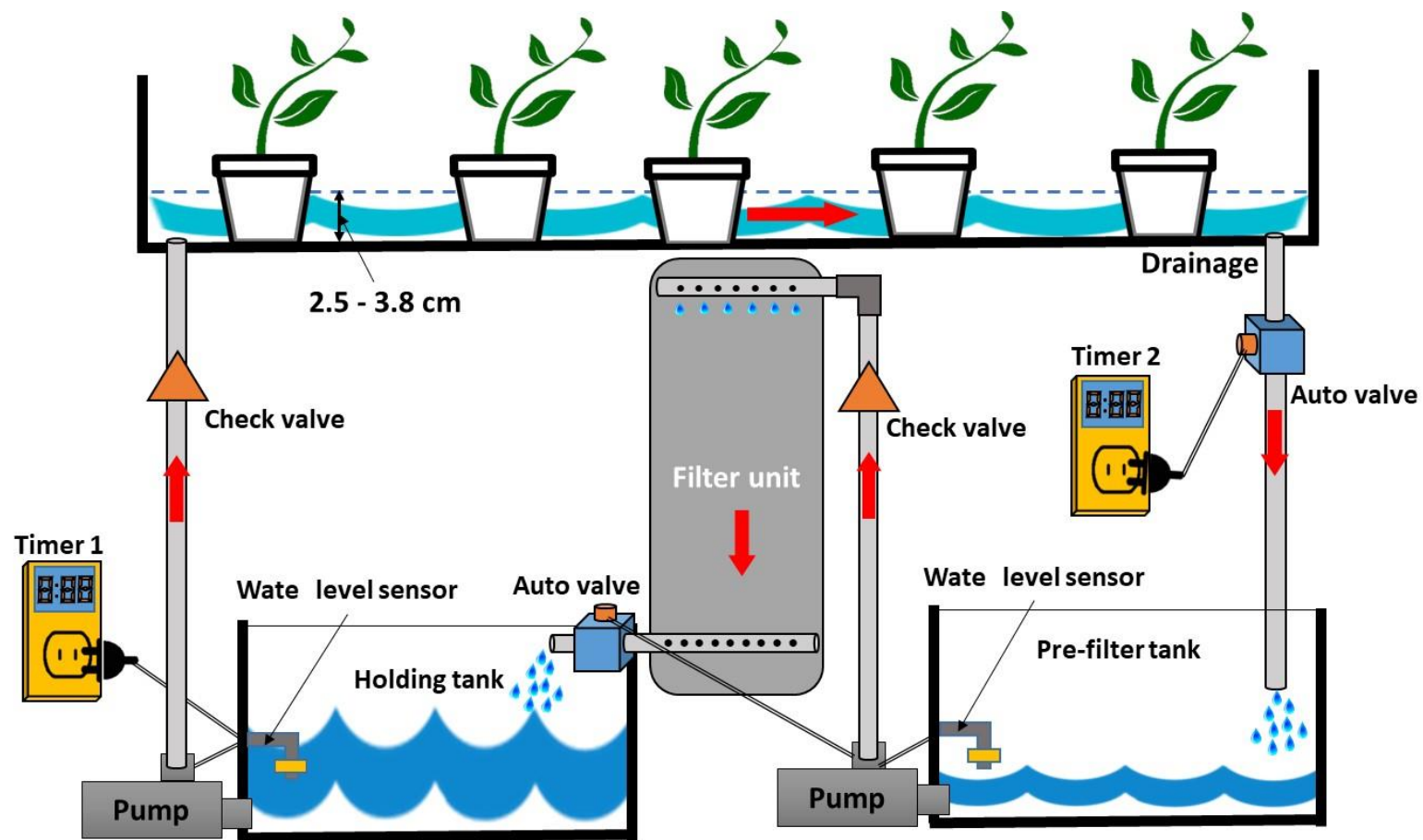


Figure 3.S1. Schematic of the ebb-and-flow irrigation system constructed in the greenhouse.

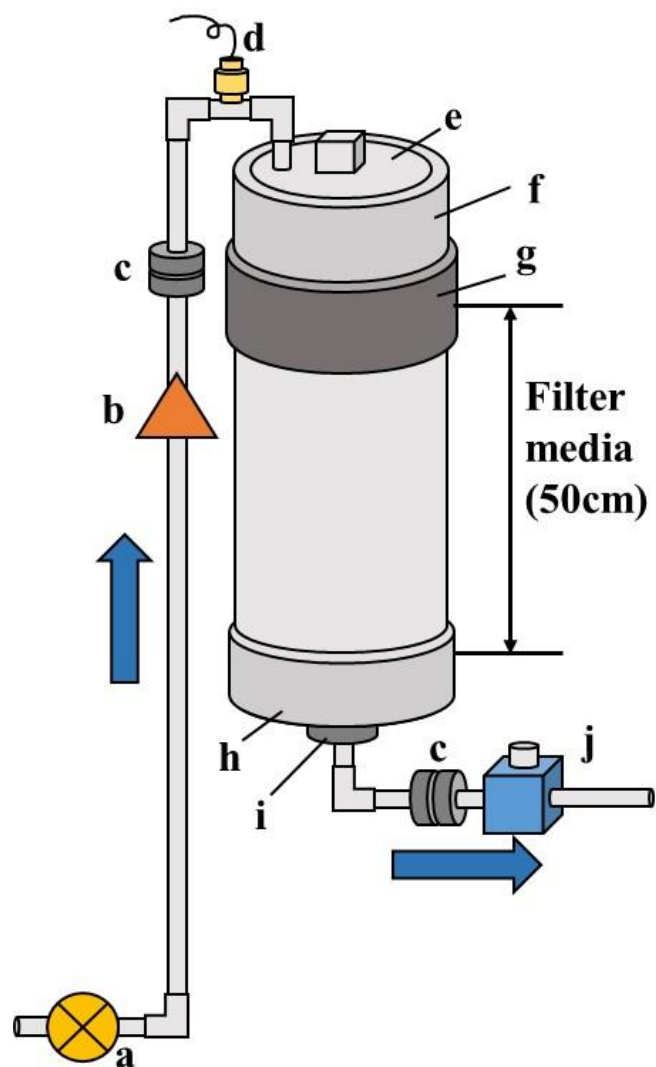


Figure 3.S2. Schematic of filter unit (a. 12-V water pump, b. check valve, c. union fitting, d: pressure sensor, e. PVC plug fitting, f. PVC adapter fitting, g. PVC coupling, h. PVC end cap fitting, i. Bulkhead fitting, and j. motorized ball valve).

S1.3. Preparation of *Pythium* Zoospores

The *Pythium* isolates were grown on the V8-agar culture for 5 days. The V8-agar culture was then divided into six strips and separated into two sterile petri dishes of 100 mm in diameter. The petri dishes were flooded with sterile distilled water (SDW), incubated at 30 °C for 24 h, drained, rinsed, and flooded with another 25 mL of SDW. After incubation for 10 h at ambient temperature ($21 \pm 2^\circ\text{C}$), the zoospores were released from the sporangia, and then were transferred to a 2-L beaker half filled with SDW. To determine the concentration of the zoospore suspension, a 1-mL aliquot was placed into a 1.7 mL microcentrifuge tube, vortexed for 70 seconds to induce the zoospore encystment, and then a 10 μL aliquot was pipetted onto a clean hemocytometer for counting (Bright-Line, Hausser Scientific, Horsham, PA).

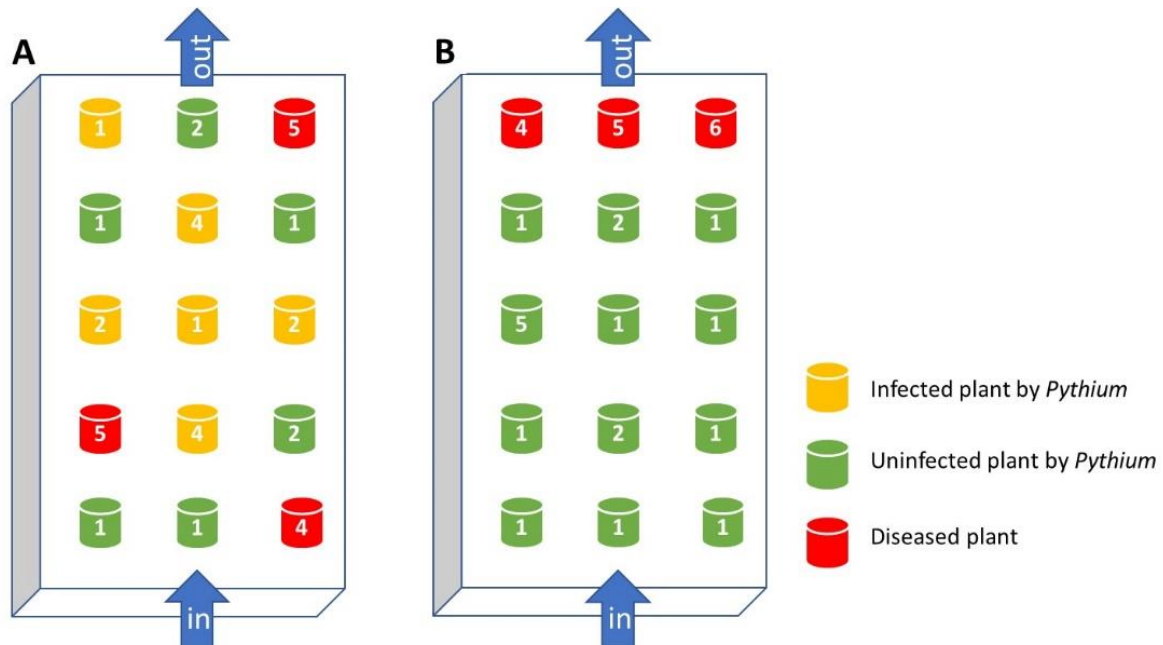


Figure 3.S3. Location of pre-inoculated plants (red), infected plant (yellow), and healthy plants (green) at the end of the first (A) and second (B) experiments. The number in the column is the root rot severity. The infection of the plants was identified by the isolation of *Pythium aphanidermatum*.

S1.4. *Pythium* Isolation

To isolate *P. aphanidermatum* from the poinsettia roots, the root mass was rinsed gently under running tap water to remove any adhering potting mixture. Three water-soaked or discolored roots were selected per plant and were surface-sterilized in 70% ethanol, blotted dry, and plated onto amended-CMA and incubated at 30°C for 24 h. *Pythium* colonies were transferred to CMA and confirmed as *P. aphanidermatum* by sporangial and oospore morphology.⁷⁷ The number of plants with the presence of *P. aphanidermatum* was divided by the total number of plants ($n = 15$) to determine the root infection ratio (IR).



Figure 3.S4. Horticultural rating scale in the second experiment (#1 = high aesthetic quality, and #5 = no aesthetic value).

S1.5. Chlorophyll Analysis

Chlorophyll a, and b concentrations in the poinsettia leaves were measured using a colorimetric method,⁴⁰ as described at the following. Three poinsettias were randomly selected and 0.5 g of the leaf sample was collected from more than 3 leaves in the middle-part of each plant. The fresh leaf samples were processed immediately after collection. The weighted leaf samples were homogenized with the addition of 10 mL of 80 % acetone. The extract was centrifuged at 2500 rpm for 5 min, and the supernatant was diluted 10 times by adding 80% acetone. The final extracted solution was analyzed for the chlorophyll concentrations by a spectrophotometer (Varian Cary 50 Bio, McKinley, New York).

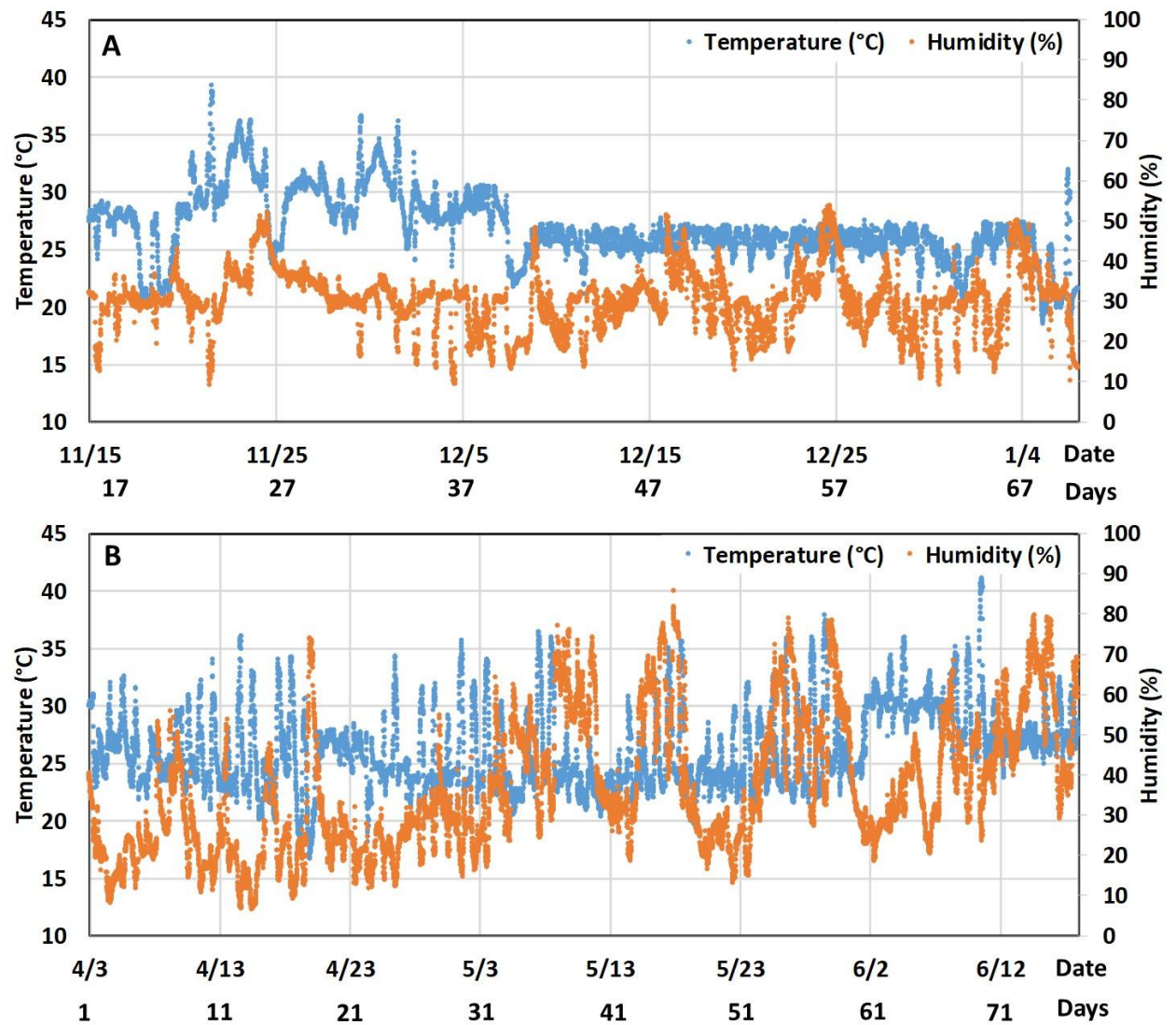


Figure 3.S5. Air temperature and relative humidity in the first (A) and second (B) experiments.

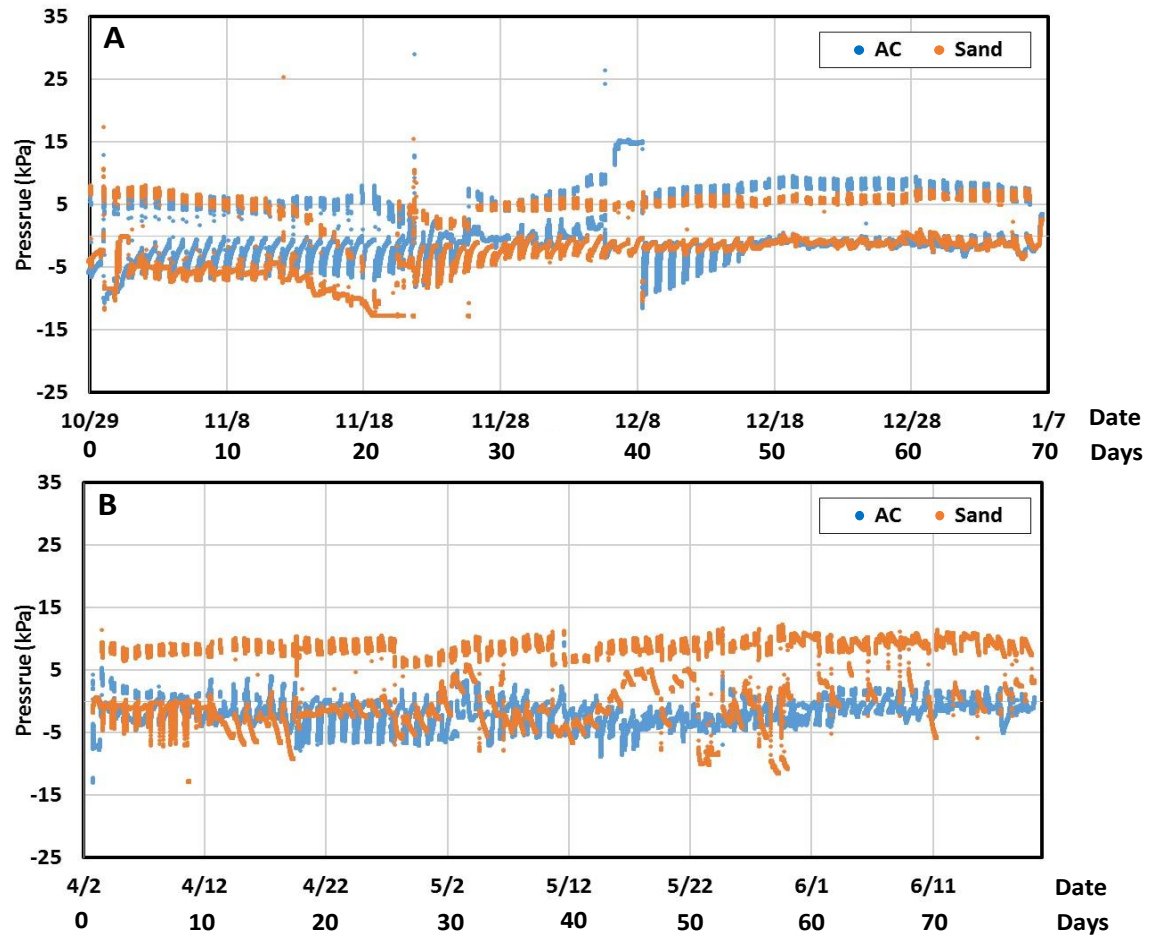


Figure 3.S6. Water pressure in the activated carbon (AC) and sand filters in the first (A) and second (B) experiments.

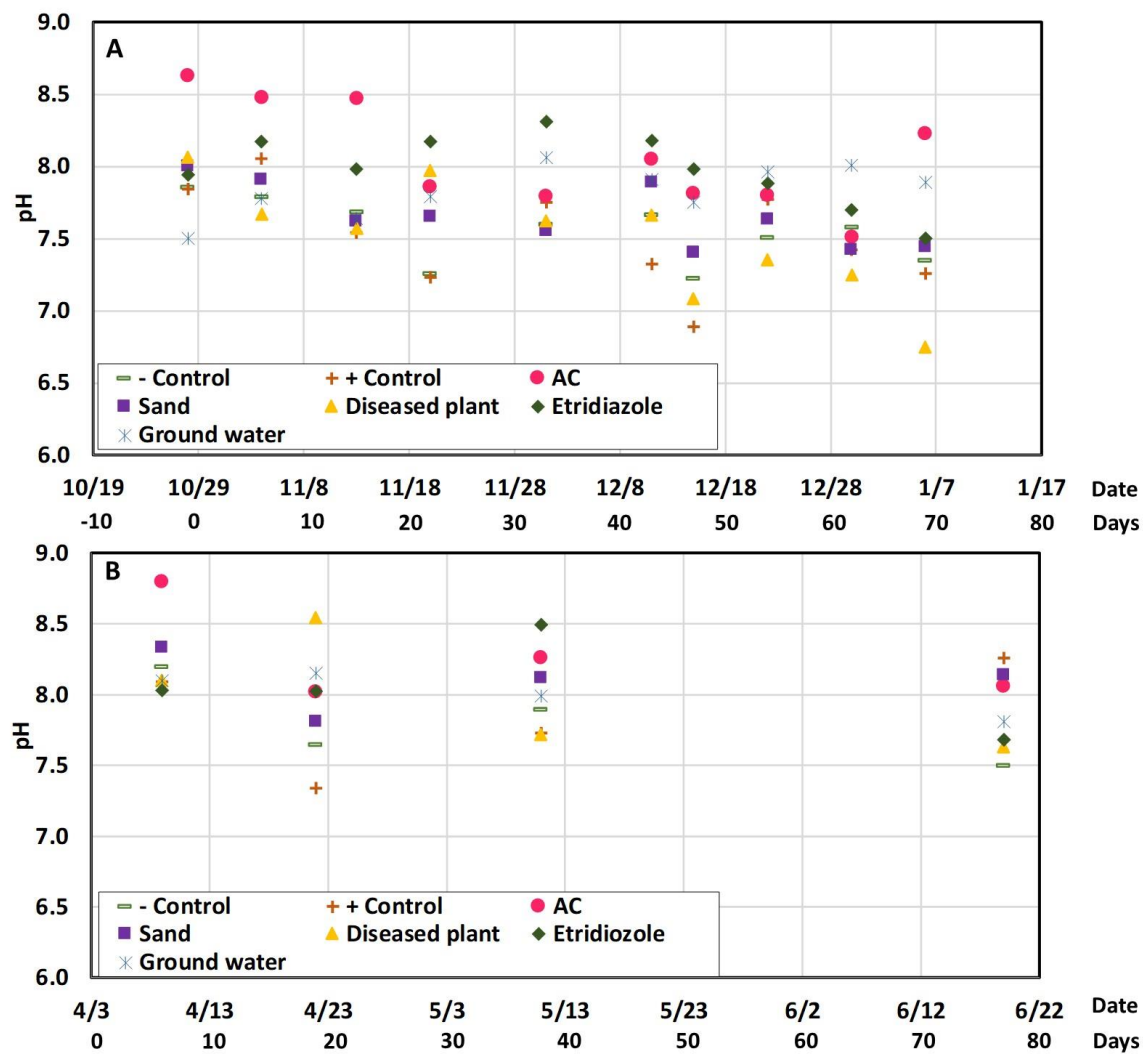


Figure 3.S7. pH of irrigation water measured in the first (A) and second (B) experiments.

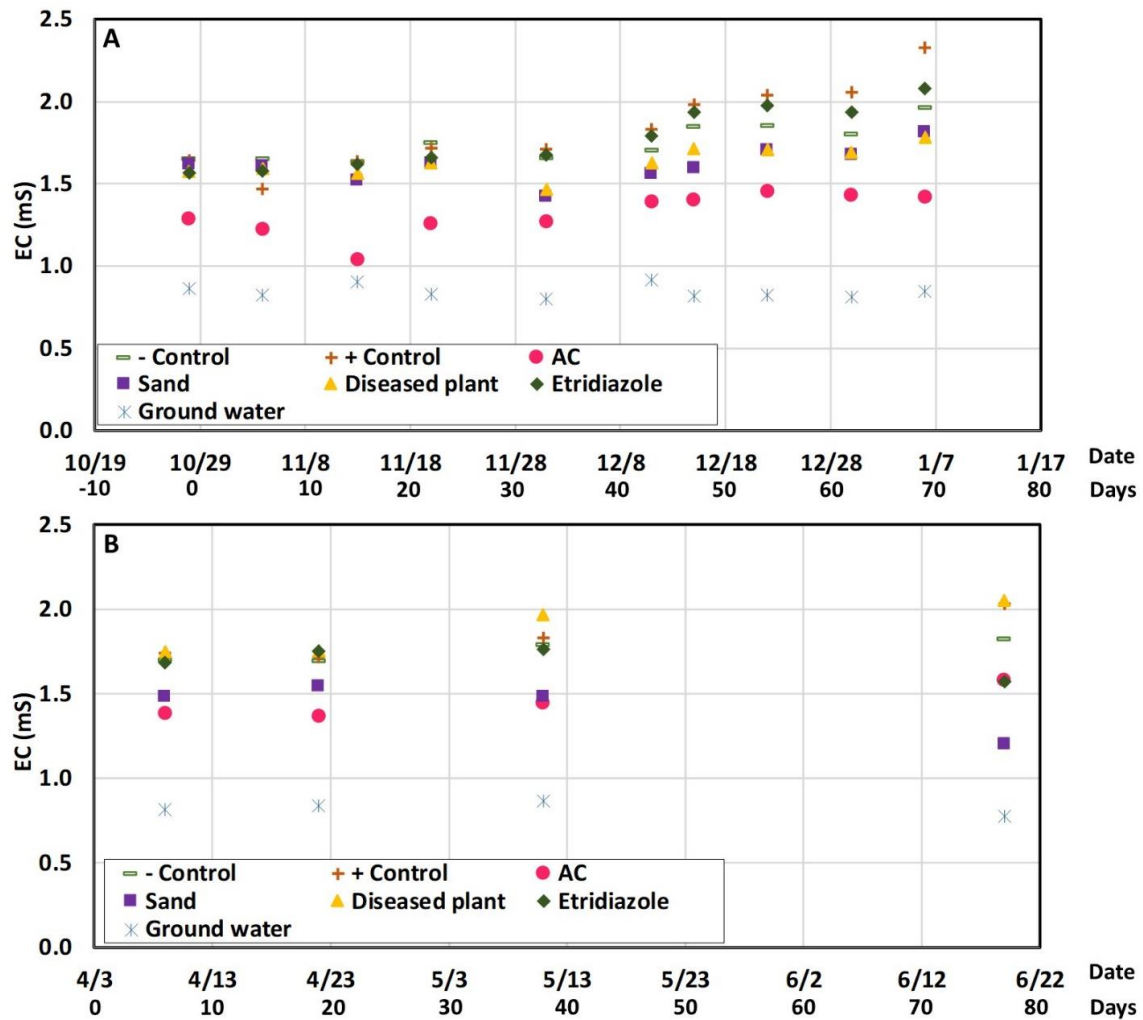


Figure 3.S8. Electrical conductivity (EC) of irrigation water measured in the first (A) and second (B) experiments.

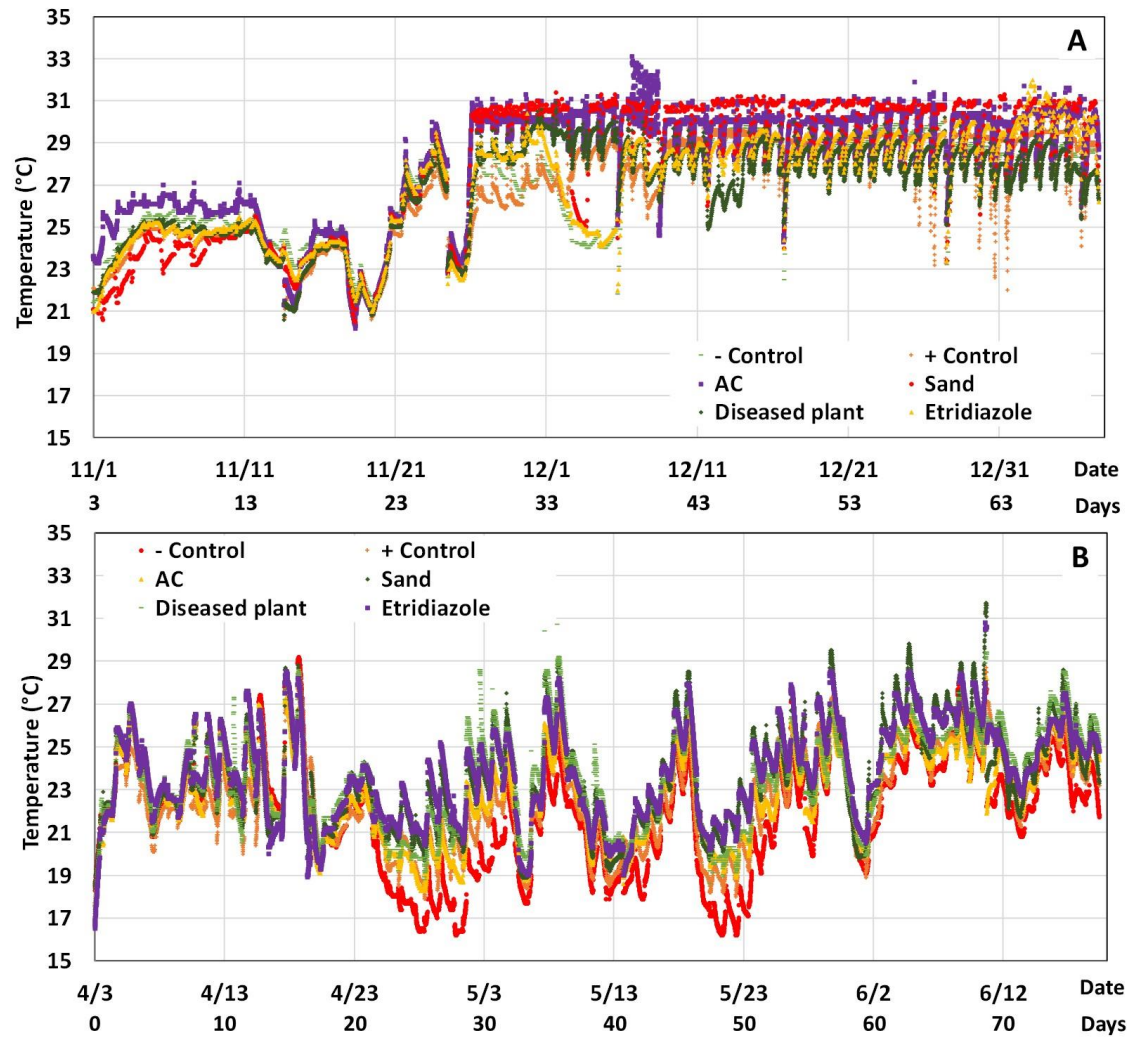


Figure 3.S9. Irrigation water temperature in the holding tank for the first (A) and second experiments (B).

Table 3.S1. Results of nutrients in the irrigation water during the first and second experiments.

Treatments		NO ₃ ⁻			P			K			Ca			Mg			Na		
Days*		0	22	69	0	22	69	0	22	69	0	22	69	0	22	69	0	22	69
1st	-Control	92	128	172	7.7	9.7	8.8	111	117	135	95	123	150	38	47	59	16	21	29
	+Control	88	128	263	7.5	6.8	8.8	100	109	149	95	123	177	41	60	73	16	22	38
	Sand	91	117	150	6.2	7	8.6	91	96	109	95	123	136	35	45	53	16	21	26
	AC	41	53	95	2.6	2.7	2.4	100	91	99	55	95	109	36	35	44	16	18	22
	Etridiazole	83	88	147	6.2	6.6	5.3	94	110	126	95	109	150	39	45	83	21	24	35
	Diseased plant	85	116	151	6.6	5.6	14.5	94	107	110	95	123	123	36	48	51	16	20	23
	Ground water	0	n.a.	n.a.	0.4	n.a.	n.a.	2	n.a.	n.a.	109	n.a.	n.a.	36	n.a.	n.a.	17	n.a.	n.a.
Days		5	38	78	5	38	78	5	38	78	5	38	78	5	38	78	5	38	78
2nd	-Control	87	154	154	5.5	6.2	6.4	96	122	125	123	150	150	44	56	57	26	30	24
	+Control	89	154	150	7.7	9.2	5.2	103	132	143	123	150	164	41	53	62	26	30	27
	Sand	62	100	63	4	3.5	2.3	91	97	54	123	123	109	42	48	44	25	26	19
	AC	36	72	94	1.8	2.2	2.6	94	109	111	68	123	136	41	51	54	25	29	27
	Etridiazole	83	98	80	6.4	2.8	4.4	103	123	100	123	109	109	45	54	47	31	36	27
	Diseased plant	88	163	163	7	4.7	3.7	106	147	147	123	164	164	45	62	63	25	31	28
	Ground water	0	n.a.	n.a.	0.1	n.a.	n.a.	2	n.a.	n.a.	95	n.a.	n.a.	35	n.a.	n.a.	15	n.a.	n.a.

* The numbers in the “days” means days calculating from the start of the experiments.

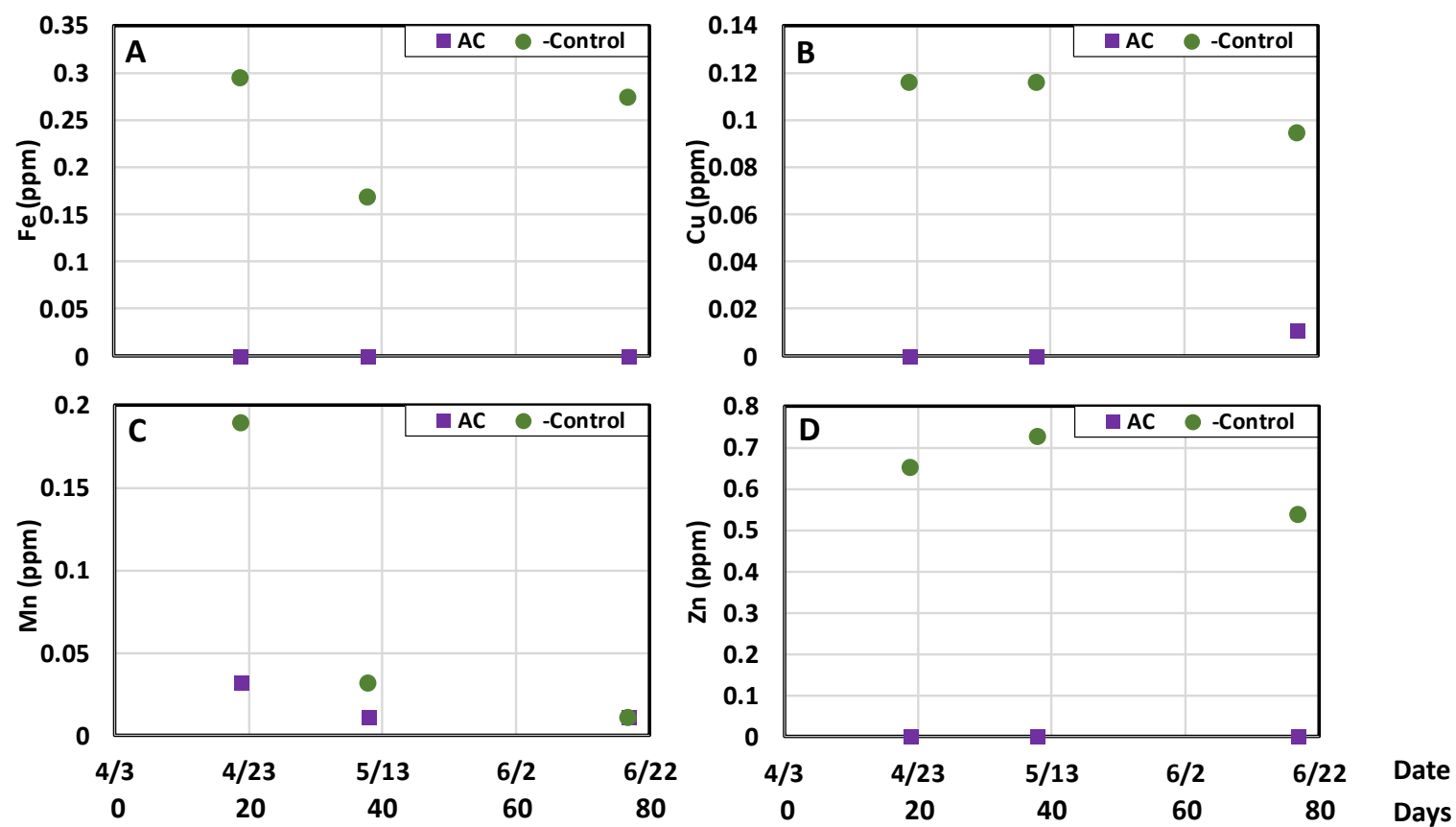


Figure 3.S10. Micronutrient concentrations in the irrigation water in the activated carbon (AC) and non-inoculated control treatments during the second experiment.



Figure 3.S11. Poinsettias at the end of the first experiment (i.e., 69 days after inoculation).

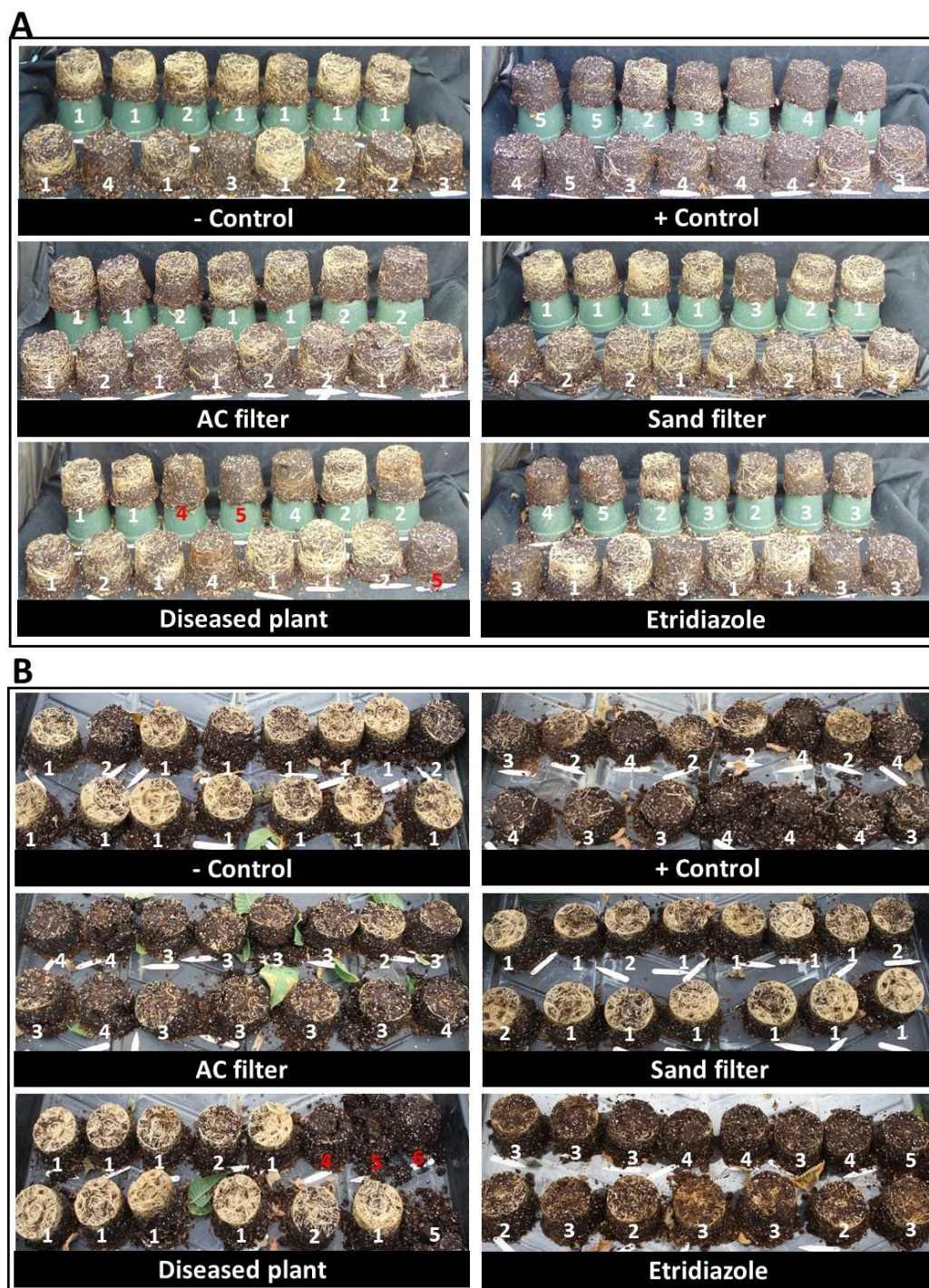


Figure 3.S12. Poinsettia roots for evaluating root necrosis in the first (A) and second (B) experiments. White color of the roots indicates a healthy root system, and dark color and dispersed potting soil suggest a rot root system. The number is the rating of root rot severity (1 = no symptoms, 2 = mild root rot, $<1/3$ affected, 3 = intermediate root rot, $1/3$ to $2/3$ affected, 4 = severe root rot, $>2/3$ roots affected, 5 = severe root and crown rot, and 6 = dead plant).

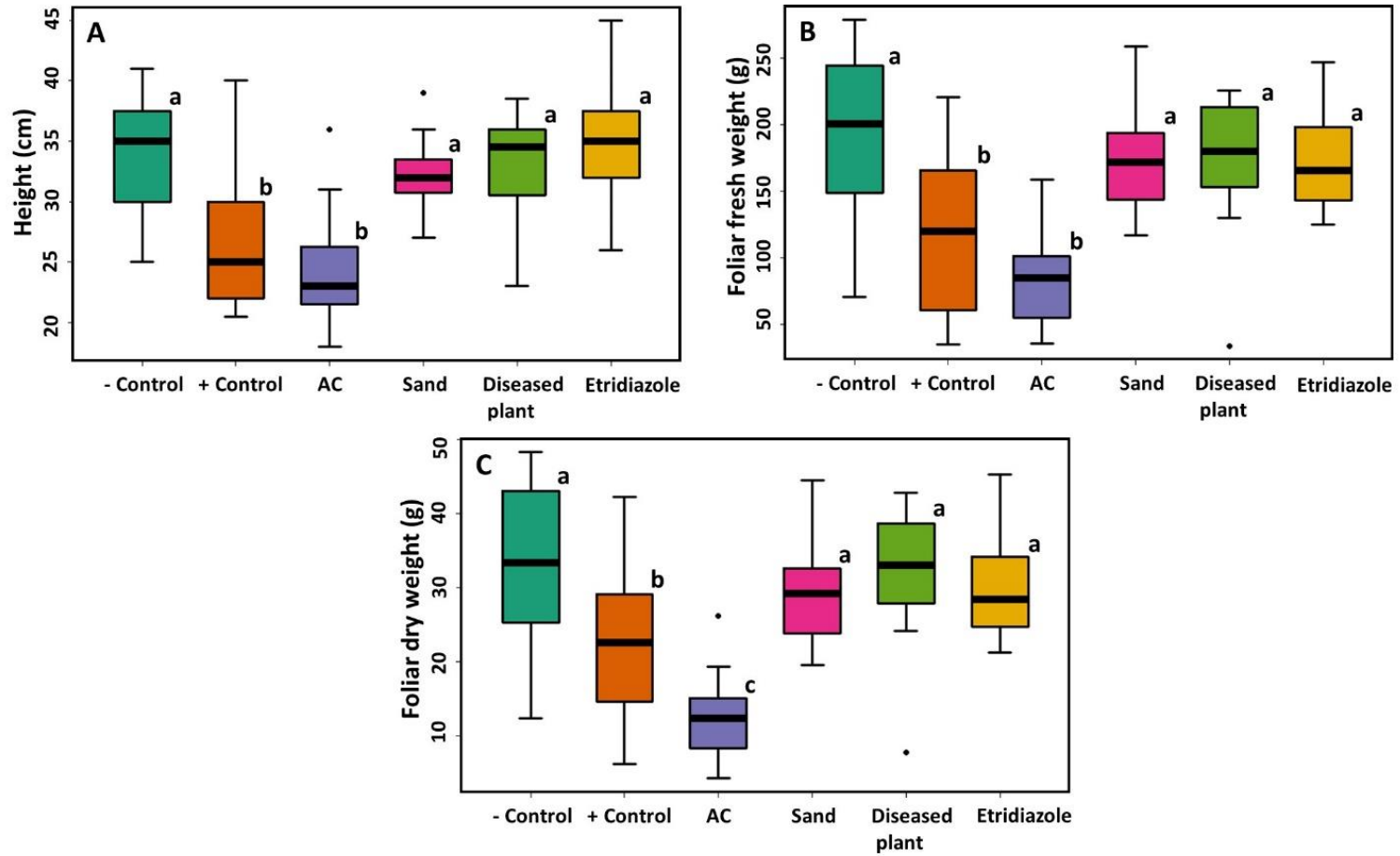


Figure 3.S13. Poinsettia height (A), foliar fresh biomass (B), and foliar dry biomass (C) in the second experiment (LSD test, $P < 0.05$).

Table 3.S2. Micronutrients in the leaves of the poinsettias in the second experiment.

	N	S	P	K	Mg	Ca	Na
	%						
AC	3.07 ± 0.21 a*	0.33 ± 0.01 a	0.52 ± 0.12 a	4.52 ± 0.51 a	0.97 ± 0.19 a	2.04 ± 0.17 a	0.030 ± 0.014 a
-Control	3.39 ± 0.02 a	0.29 ± 0.01 b	0.41 ± 0.02 a	3.57 ± 0.04 a	0.68 ± 0.06 a	1.55 ± 0.03 b	0.013 ± 0.005 a
	B	Zn	Mn	Fe	Cu	Al	
	mg/kg						
AC	14.7 ± 4.7 a	31.3 ± 4.2 a	129.7±16.7 a	44.0±8.3 a	2.0±0.8 a	41.7±13.3 a	
-Control	41.7 ± 2.9 b	59.3 ± 3.3 b	95.7±7.6 a	74.0±3.3 b	5.0±0.8 b	39.3±5.4 a	

*t-test, $P < 0.05$

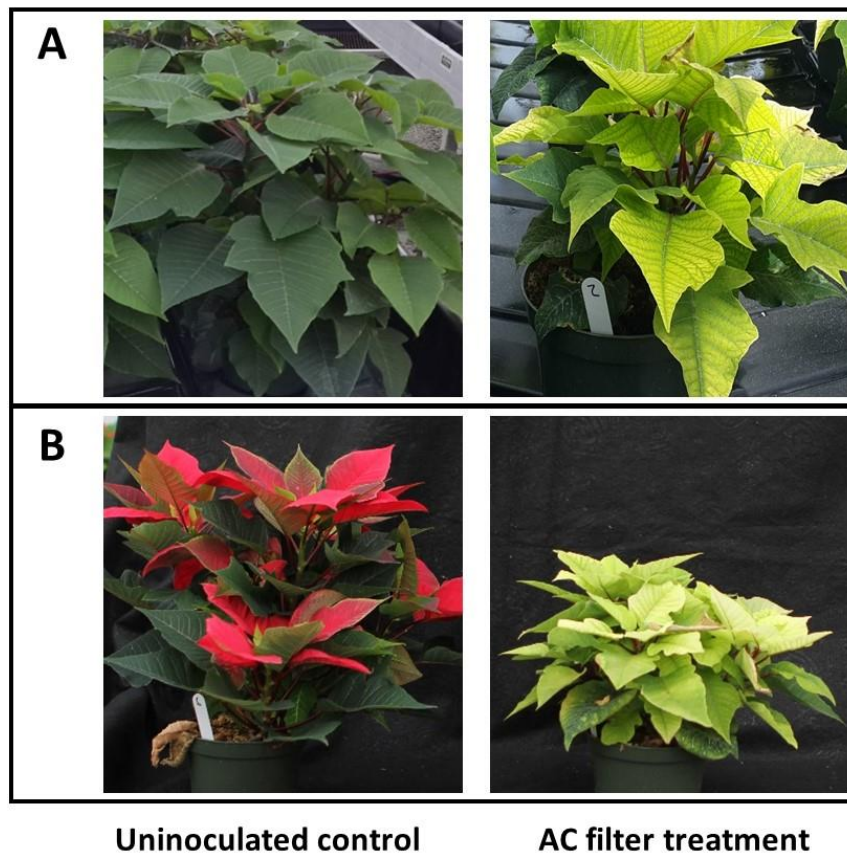


Figure 3.S14. Color of leaves comparison between non-inoculated control and AC filter treatments in the first (A) and second (B) experiments.

S1. Poinsettia Greenhouse Experiments Raw Data

Table 3.S3. Experiment A: Poinsettia Foliar fresh weight (g)

Number	- Control	+ Control	AC filter	Sand filter	Diseased plant	Etridiazole
1	219	88	159	173	138	160
2	198	103	150	179	246	123
3	188	157	149	195	I.P ^c	197
4	185	125	145	164	I.P	171
5	201	136	177	115	152	202
6	186	102	115	200	186	131
7	191	110	133	147	175	156
8	193	132	165	216	169	174
9	118	112	161	146	223	226
10	165	96	135	195	176	196
11	151	114	138	188	180	176
12	204	94	184	178	215	207
13	187	77	149	166	183	173
14	96	118	121	178	214	163
15	90	105	170	182	I.P	156
Mean	171.5	111.3	150.1	174.8	188.1	174.1
SD	38.56	19.69	19.08	24.2	29.66	27.10

^c I.F = pre-infected plant = biomass of initially infected plant was not included for data analysis.

^d Plants were cut and used as propagation for second experiment after fresh weight measurement.

Table 3.S4. Experiment A: Poinsettia foliar dry weight (g)

Number	- Control	+ Control	AC filter	Sand filter	Diseased plant	Etridiazole
1	26.5	12.5	20.8	N.A	16.9	20.6
2	23.7	14.0	18.3	22.1	30.0	16.6
3	25.3	20.4	18.2	24.3	I.P	N.A
4	22.2	16.5	N.A	19.8	I.P	22.0
5	25.5	17.2	21.2	15.0	20.7	25.7
6	N.A ^d	13.6	13.8	25.9	N.A	16.8
7	23.2	15.3	16.1	17.3	N.A	20.5
8	N.A	18.5	20.0	27.8	N.A	N.A
9	16.4	15.1	19.6	N.A	27.0	29.2
10	20.1	13.1	N.A	24.8	21.6	25.1
11	N.A	14.4	16.8	23.8	23.7	N.A
12	25.9	13.0	23.0	22.8	26.0	26.4
13	27.9	10.0	18.1	N.A	22.7	21.8
14	N.A	15.4	15.0	23.1	26.0	21.8
15	13.0	13.4	19.4	24.6	I.P	20.9
Mean	22.7	14.8	18.5	22.6	23.8	22.3
SD	4.3	2.5	2.5	3.5	3.7	3.6

Table 3.S5. Experiment A: Poinsettia root dry weight (g)

Number	- Control	+ Control	AC filter	Sand filter	Diseased plant	Etridiazole
1	5.6	2.5	3.3	5.9	3.4	4.1
2	5.6	2.9	2.9	4.5	6.5	2.8
3	7.0	3.8	3.3	7.7	I.P	6.3
4	4.6	2.8	3.9	3.6	I.P	3.9
5	6.2	3.2	2.9	2.5	4.7	4.6
6	4.1	1.8	3.5	4.8	5.6	6.1
7	5.0	2.8	2.2	3.6	5.0	3.5
8	7.1	3.5	3.3	3.1	4.7	5.1
9	3.3	2.3	4.2	4.3	4.6	6.9
10	3.2	2.8	3.2	5.4	4.7	6.1
11	3.8	2.5	3.0	6.0	4.6	3.5
12	4.9	2.5	3.8	6.0	5.1	6.1
13	4.9	1.4	3.9	2.8	5.4	5.7
14	2.8	2.3	2.7	4.2	5.7	3.9
15	2.0	2.9	2.7	7.5	I.P	4.7
Mean	4.7	2.7	3.2	4.8	5.0	4.9
SD	1.4	0.6	0.5	1.6	0.7	1.2

Table 3.S6. Experiment A: Poinsettia height (cm)

Number	- Control	+ Control	AC filter	Sand filter	Diseased plant	Etridiazole
1	35	25	31	31.5	27.5	30
2	38	27	28	31	36.5	28.5
3	35.5	32	27.5	36	I.P	34
4	33	26	28.5	31	I.P	36
5	34	33	30	32	31.5	39.5
6	34	24	31	38	33	33
7	32	27	25.5	35	29.5	27
8	35.5	28	29.5	38.5	32	33
9	29	27	35	31	32.5	36
10	31	26.5	31	36.5	34.5	38
11	33	24.5	33	38	35	36
12	33	24	35	37	36	36.5
13	35.5	19	34	34	37	32.5
14	30	25.5	29.5	33.5	35	34
15	24	27	32	40	I.P	33
Mean	32.8	26.4	30.7	34.9	33.3	33.8
SD	3.3	3.2	2.7	3.0	2.8	3.3

Table 3.S7. Experiment A: Poinsettia root rot severity

Number	- Control	+ Control	AC filter	Sand filter	Diseased plant	Etridiazole
1	1	5	1	1	1	4
2	1	5	1	1	1	5
3	2	2	2	1	I.P	2
4	1	3	1	1	I.P	3
5	1	5	1	3	4	2
6	1	4	2	2	2	3
7	1	4	2	1	2	3
8	1	4	1	4	1	3
9	4	5	2	2	2	1
10	1	3	1	2	1	1
11	3	4	1	1	4	3
12	1	4	2	1	1	1
13	2	4	2	2	1	1
14	2	2	1	1	2	3
15	3	3	1	2	I.P	3
Mean	1.7	3.8	1.4	1.7	1.8	2.5
SD	0.94	0.98	0.49	0.87	1.07	1.15

Table 3.S8. Experiment A: Poinsettia chlorophyll a and b

	- Control	+ Control	AC filter	Sand filter	Diseased plant	Etridiazole
Chlorophyll a	41.5	51.0	18.1	38.2	48.2	46.1
	42.5	47.5	16.4	43.3	47.6	43.7
	44.1	48.8	15.0	35.9	47.9	48.6
Chlorophyll b	71.5	95.5	28.7	67.0	76.9	74.2
	77.7	87.9	26.1	76.3	82.9	74.8
	79.7	84.8	23.2	60.9	82.1	81.8

continue

Table 3.S9. Experiment B: Poinsettia foliar fresh weight (g)

Number	- Control	+ Control	AC filter	Sand filter	Diseased plant	Etridiazole
1	203	120	135	179	224	182
2	156	124	47	184	145	176
3	277	221	54	157	223	160
4	201	35	108	182	203	247
5	153	47	82	170	186	204
6	252	51	93	147	175	148
7	208	176	36	259	34	199
8	145	54	41	172	172	227
9	71	155	107	117	185	166
10	279	189	56	207	162	125
11	133	97	159	119	130	135
12	159	68	96	204	226	139
13	249	147	76	244	I.P	131
14	240	214	89	141	I.P	165
15	140	96	85	129	I.P	198
Mean	191.1	119.6	84.3	174.1	172.1	173.5
SD	58.50	60.21	33.52	40.60	50.98	34.94

Table 3.S10. Experiment B: Poinsettia foliar dry weight (g)

Number	- Control	+ Control	AC filter	Sand filter	Diseased plant	Etridiazole
1	35.1	22.8	19.4	32.5	41.2	31.5
2	28.2	22.5	6.5	30.9	24.8	32.1
3	48.3	42.3	8.0	25.6	41.9	27.3
4	33.4	6.2	15.2	31.6	36.1	45.3
5	24.8	19.0	11.3	27.9	33.0	34.6
6	45.0	9.6	14.8	25.9	33.5	25.2
7	37.3	31.6	4.2	44.5	7.7	33.7
8	25.5	10.3	6.3	29.2	32.8	39.6
9	12.4	26.6	17.1	19.5	33.1	28.5
10	46.9	34.4	8.6	34.1	30.9	21.2
11	21.9	17.1	26.1	20.3	24.2	22.6
12	26.8	12.1	13.9	32.6	42.9	24.1
13	42.5	26.4	11.1	41.8	I.P	22.1
14	43.5	36.9	12.4	22.0	I.P	27.6
15	25.0	18.0	14.1	21.2	I.P	34.7
Mean	33.1	22.4	12.6	29.3	31.8	30.0
SD	10.31	10.35	5.49	7.13	9.24	6.62

Table 3.S11. Experiment B: Poinsettia height (cm)

Number	- Control	+ Control	AC filter	Sand filter	Diseased plant	Etridiazole
1	36	22	26.5	34	33.5	31.5
2	29	20.5	23	36	35	33
3	35	31	18	32	37	32
4	40	21	27.5	33	34	45
5	32	22	23	30	31	33
6	31	23	26	35	38	30.5
7	38	32.5	22	39	23	36
8	28	22.5	21	33	35	44
9	25	29	25	32	30	45
10	39	32	23	31.5	28	26
11	34	26	36	27	35	35
12	36	22	24.5	32	38.5	35
13	37	27	31	30.5	I.P	37
14	41	40	20.5	30	I.P	38
15	28	25	21	31	I.P	32
Mean	33.9	26.4	24.5	32.4	29.7	35.5
SD	4.73	5.37	4.36	2.76	8.19	5.36

Table 3.S12. Experiment B: Poinsettia root rot severity

Number	- Control	+ Control	AC filter	Sand filter	Diseased plant	Etridiazole
1	1	4	3	2	1	2
2	1	3	4	1	1	3
3	1	3	3	1	1	2
4	1	4	3	1	1	3
5	1	4	3	1	2	3
6	1	4	3	1	1	2
7	1	3	4	1	5	3
8	1	3	4	1	1	3
9	2	2	4	2	1	3
10	1	4	3	1	1	3
11	1	2	3	1	2	4
12	1	2	3	1	1	4
13	1	4	3	1	I.P	3
14	1	2	2	1	I.P	4
15	2	4	3	2	I.P	3
Mean	1.1	3.2	3.2	1.2	1.5	3.0
SD	0.34	0.83	0.54	0.40	1.12	0.63

Table 3.S13. Experiment B: Poinsettia horticultural rank

Number	- Control	+ Control	AC filter	Sand filter	Diseased plant	Etridiazole
1	1	3	5	2	3	2
2	2	3	5	1	2	2
3	1	2	5	2	2	2
4	1	4	5	2	3	2
5	1	4	5	2	2	2
6	1	4	4	2	2	1
7	2	3	5	1	4	2
8	2	3	4	1	2	2
9	2	2	5	2	1	1
10	1	2	5	2	2	3
11	1	3	4	2	2	2
12	1	4	5	2	2	2
13	1	3	5	2	I.P	3
14	1	2	4	2	I.P	2
15	3	3	5	1	I.P	1
Mean	1.4	3.0	4.7	1.7	2.3	1.9
SD	0.61	0.73	0.44	0.44	0.72	0.57

Table 3.S14. Experiment B: Poinsettia chlorophyll a and b (mg/ml)

	- Control	+ Control	AC filter	Sand filter	Diseased plant	Etridiazole
Chlorophyll a	14.1	11.5	1.0	13.0	16.5	17.3
	17.6	15.3	5.1	14.2	14.9	16.4
	17.0	12.7	6.1	11.7	13.8	18.3
Chlorophyll b	98.7	80.5	6.8	87.8	112.2	116.9
	120.4	102.6	32.4	96.7	101.3	110.4
	112.1	90.1	36.9	81.3	92.1	124.5

continue

REFERENCES

REFERENCES

1. USDA Floriculture crops 2015 summary. <http://usda.mannlib.cornell.edu/usda/current/FlorCrop/FlorCrop-04-26-2016.pdf> (April 29),
2. Krasnow, C. S.; Hausbeck, M. K., Influence of pH and Etridiazole on Pythium Species. *HortTechnology* **2017**, 27, (3), 367-374.
3. Bush, E. A.; Hong, C.; Stromberg, E. L., Fluctuations of *Phytophthora* and *Pythium* spp. in components of a recycling irrigation system. *Plant Dis.* **2003**, 87, (12), 1500-1506.
4. Hong, C.; Richardson, P.; Kong, P.; Bush, E., Efficacy of chlorine on multiple species of *Phytophthora* in recycled nursery irrigation water. *Plant Dis* **2003**, 87, (10), 1183-1189.
5. MacDonald, J.; Ali-Shtayeh, M.; Kabashima, J.; Stites, J., Occurrence of Phytophthora species in recirculated nursery irrigation effluents. *Plant Dis* **1994**, 78, (6), 607-611.
6. Sanogo, S.; Moorman, G. W., Transmission and control of *Pythium aphanidermatum* in an ebb-and-flow subirrigation system. *Plant Dis.* **1993**, 77, (3), 287-290.
7. Bush, E. A.; Hong, C.; Stromberg, E. L., Fluctuations of *Phytophthora* and *Pythium* spp. in components of a recycling irrigation system. *Plant Dis.* **2003**, 87, (12), 1500-1506.
8. Meador, D. P.; Fisher, P. R.; Harmon, P. F.; Peres, N. A.; Teplitski, M.; Guy, C. L., Survey of physical, chemical, and microbial water quality in greenhouse and nursery irrigation water. *HortTechnology* **2012**, 22, (6), 778-786.
9. Van der Gaag, D. J.; Keressies, A.; Lanser, C., Spread of phytophthora root and crown rot in saintpaulia, gerbera and spathiphyllum pot plants in ebb-and-flow-systems. *European J. Plant Pathol.* **2001**, 107, (5), 535-542.
10. Ehret, D.; Alsanius, B.; Wohanka, W.; Menzies, J.; Utkhede, R., Disinfestation of recirculating nutrient solutions in greenhouse horticulture. *Agronomie* **2001**, 21, (4), 323-339.
11. Evans, S., Susceptibility of plants to fungal pathogens when grown by the nutrient–film technique (NFT). *Plant Pathology* **1979**, 28, (1), 45-48.
12. Goldberg, N.; Stanghellini, M.; Rasmussen, S., Filtration as a method for

- controlling *Pythium* root rot of hydroponically grown cucumbers. *Plant Dis* **1992**, 76, (8), 777-779.
13. Stanghellini, M.; Kim, D.; Rasmussen, S.; Rorabaugh, P. A., Control of root rot of peppers caused by *Phytophthora capsici* with a nonionic surfactant. *Plant Dis* **1996**, 80, (10), 1113-1116.
 14. Stanghellini, M.; Rasmussen, S.; Kim, D.; Rorabaugh, P. A., Efficacy of nonionic surfactants in the control of zoospore spread of *Pythium aphanidermatum* in a recirculating hydroponic system. *Plant Dis* **1996**, 80, (4), 422-428.
 15. Hong, C. X.; Moorman, G. W., Plant pathogens in irrigation water: Challenges and opportunities. *Crit. Rev. Plant Sci.* **2005**, 24, (3), 189-208.
 16. Tompkins, C. M.; Middleton, J. T., Etiology and control of poinsettia root and stem rot caused by *Pythium* spp. and *Rhizoctonia solani*. *Hilgardia* **1950**, 20, 171-82.
 17. Stephens, C.; Herr, L.; Schmitthenner, A.; Powell, C., Sources of *Rhizoctonia solani* and *Pythium* spp. in a bedding plant greenhouse. *Plant Dis.* **1983**, 67, (3), 272-275.
 18. Elmer, W.; Gent, M.; McAvoy, R., Partial saturation under ebb and flow irrigation suppresses *pythium* root rot of ornamentals. *Crop Prot* **2012**, 33, 29-33.
 19. Oh, M.; Son, J. E., *Phytophthora nicotianae* transmission and growth of potted kalanchoe in two recirculating subirrigation systems. *Scientia horticultrae* **2008**, 119, (1), 75-78.
 20. Hoitink, H. A. J., Transmission of plant pathogens in an ebb and flood system. *Ohio Florist Assn. Bul.* **1991**, 742, 5-9.
 21. Moorman, G.; Kim, S., Species of *Pythium* from greenhouses in Pennsylvania exhibit resistance to propamocarb and mefenoxam. *Plant Dis.* **2004**, 88, (6), 630-632.
 22. Raabe, R.; Hurlimann, J.; Byrne, T., Chemical and biological approaches to control of *pythium* root rot of poinsettia. *California Plant Pathol.* **1981**, 52, 5-8.
 23. Ascerno, M. E.; Pfleger, F.; Wilkins, H. F., Effect of root rot and *Rhizoglyphus robini* on greenhouse-forced easter lily development. *Environmental Entomology* **1981**, 10, (6), 947-949.
 24. Hausbeck, M. K.; Harlan, B. R., Evaluation of fungicides for control of *pythium* root rot of poinsettia, 2011. *Plant Dis. Mgmt. Rept.* **2013**, 7:OT029.
 25. CDMS Label Database. <http://www.cdms.net/Label-Database> (Last accessed on April 29, 2017),

26. Moorman, G.; Kang, S.; Geiser, D.; Kim, S., Identification and characterization of *Pythium* species associated with greenhouse floral crops in Pennsylvania. *Plant Dis.* **2002**, 86, (11), 1227-1231.
27. Lookabaugh, E. C.; Ivors, K. L.; Shew, B. B., Mefenoxam Sensitivity, Aggressiveness, and Identification of *Pythium* Species Causing Root Rot on Floriculture Crops in North Carolina. *Plant Dis.* **2015**, 99, (11), 1550-1558.
28. Hausbeck, H.; Harlan, B., *Pythium* root rot in the greenhouse. 8 Sep. 2016. <http://msue.anr.msu.edu/news/pythium_root_rot_in_the_greenhouse> **2013**.
29. Carlson, K.; Hong, C.; Richardson, P., Characterization of fungicide resistance and pathogenic fitness in a population of *Pythium dissotocum* isolated from nursery irrigation water. *Phytopathology* **2004**, 94, (6), S14.
30. Hausbeck, M.; Zhang, W., Prevent the spread of disease in irrigation water. *Greenhouse Grower*. **2016**, Mar, 68-70.
31. Schuerger, A. C.; Hammer, W., Use of cross-flow membrane filtration in a recirculating hydroponic system to suppress root disease in pepper caused by *Pythium myriotylum*. *Phytopathology* **2009**, 99, (5), 597-607.
32. Tu, J.; Harwood, B., Disinfestation of recirculating nutrient solution by filtration as a means to control *Pythium* root rot of tomatoes. *Acta Horticulturae* **2005**, 695, 303-307.
33. Erwin, D. C.; Bartnicki-Garcia, S.; Tsao, P., *Phytophthora: Its Biology, Ecology and Pathology*. American Phytopathological Society, St. Paul, MN: 1983.
34. Darling, D., A mobile filtration plant to eliminate fungal spores in irrigation water for plant propagation. Short communication. *Australian Forest Research* **1977**, 7, 273-274.
35. Lee, E.; Oki, L. R., Slow sand filters effectively reduce *Phytophthora* after a pathogen switch from *Fusarium* and a simulated pump failure. *Water Res* **2013**, 47, (14), 5121-5129.
36. Quesada-Ocampo, L. M.; Hausbeck, M. K., Resistance in tomato and wild relatives to crown and root rot caused by *Phytophthora capsici*. *Phytopathology* **2010**, 100, 619-627.
37. Rahimian, M.; Banihashemi, Z., A method for obtaining zoospores of *Pythium aphanidermatum* and their use in determining cucurbit seedling resistance to damping-off. *Plant Dis. Rptr.* **1979**, 63, (8), 658-661.
38. Ecke III, P.; Faust, J. E.; Higgins, A.; Williams, J., *The Ecke poinsettia manual*. Ball publishing: 2004.

39. Boehm, M.; Hoitink, H., Sustenance of microbial activity in potting mixes and its impact on severity of *Pythium* root rot of poinsettia. *Phytopathology* **1992**, 82, (3), 259-264.
40. Wellburn, A. R., The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *Journal of plant physiology* **1994**, 144, (3), 307-313.
41. Gadgil, A., Drinking water in developing countries. *Annual review of energy and the environment* **1998**, 23, (1), 253-286.
42. Huisman, L.; Wood, W., *Slow sand filtration*. World Health Organization Geneva: 1974; Vol. 16.
43. WHO Rapid Sand Filtration. (Fact Sheets on Environmental Sanitation). http://www.who.int/water_sanitation_health/hygiene/emergencies/fs2_14.pdf (May 27),
44. Vankuik, A., Eliminating *Phytophthora cinnamomi* in a recirculated irrigation system by slow sand filtration. *Mededelingen-Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen Universiteit Gent (Belgium)* **1994**, 59, 1059-1063.
45. Mizuta, K.; Matsumoto, T.; Hatate, Y.; Nishihara, K.; Nakanishi, T., Removal of nitrate-nitrogen from drinking water using bamboo powder charcoal. *Bioresource Technol* **2004**, 95, (3), 255-257.
46. Ozturk, N.; Bektas, T. E., Nitrate removal from aqueous solution by adsorption onto various materials. *J Hazard Mater* **2004**, 112, (1-2), 155-62.
47. Ganrot, Z.; Dave, G.; Nilsson, E., Recovery of N and P from human urine by freezing, struvite precipitation and adsorption to zeolite and active carbon. *Bioresour Technol* **2007**, 98, (16), 3112-21.
48. Li, Y.; Liu, C.; Luan, Z.; Peng, X.; Zhu, C.; Chen, Z.; Zhang, Z.; Fan, J.; Jia, Z., Phosphate removal from aqueous solutions using raw and activated red mud and fly ash. *J Hazard Mater* **2006**, 137, (1), 374-83.
49. Zhang, L.; Wan, L.; Chang, N.; Liu, J.; Duan, C.; Zhou, Q.; Li, X.; Wang, X., Removal of phosphate from water by activated carbon fiber loaded with lanthanum oxide. *J Hazard Mater* **2011**, 190, (1-3), 848-55.
50. Jeon, S.; Krasnow, C. S.; Kirby, C. K.; Granke, L. L.; Hausbeck, M. K.; Zhang, W., Transport and Retention of *Phytophthora capsici* Zoospores in Saturated Porous Media. *Environmental Science & Technology* **2016**, 50, (17), 9270-9278.
51. Darling, D., A mobile filtration plant to eliminate fungal spores in irrigation water for plant propagation. Short communication. *Australian Forest Research* **1977**.

52. Vankuik, A., Eliminating Phytophthora cinnamomi in a recirculated irrigation system by slow sand filtration. *Mededelingen-Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen Universiteit Gent (Belgium)* **1994**.
53. Déniel, F.; Rey, P.; Chérif, M.; Guillou, A.; Tirilly, Y., Indigenous bacteria with antagonistic and plant-growth-promoting activities improve slow-filtration efficiency in soilless cultivation. *Canadian journal of microbiology* **2004**, 50, (7), 499-508.
54. van Os, E. A.; Amsing, J. J.; van Kuik, A. J.; Willers, H., Slow sand filtration: A potential method for the elimination of pathogens and nematodes in recirculating nutrient solutions from glasshouse-grown crops. *International Symposium on Growing Media and Hydroponics, Vols I and II* **1998**, (481), 519-526.
55. Ufer, T., Werres, S. K., Posner, M., and Wessels, H.-P., Filtration to eliminate Phytophthora spp. from recirculating water systems in commercial nurseries. *Online Plant Health Progress* **2008**, <http://dx.doi.org/10.1094/PHP-2008-0314-01-RS>.
56. Van Os, E.; Bruins, M.; Wohanka, W.; Seidel, R. In *Slow filtration: a technique to minimise the risks of spreading root-infecting pathogens in closed hydroponic systems*, V International Symposium on Protected Cultivation in Mild Winter Climates: Current Trends for Sustainable Technologies 559, 2000; 2000; pp 495-502.
57. Wohanka, W.; Helle, M., Suitability of various filter media for slow filtration. *Proceedings of the 9th International Congress on Soil-less Culture, Jersey, United Kingdom. ISOSC, Wageningen, The Netherlands* **1996**, 551-557.
58. Kim, H.-S.; Lee, S.-H.; Byun, Y.; Park, H.-D., 6-Gingerol reduces Pseudomonas aeruginosa biofilm formation and virulence via quorum sensing inhibition. *Scientific reports* **2015**, 5, 8656.
59. Algburi, A.; Comito, N.; Kashtanov, D.; Dicks, L. M.; Chikindas, M. L., Control of biofilm formation: antibiotics and beyond. *Applied and environmental microbiology* **2016**, AEM. 02508-16.
60. Mosaic Diagnosis of Nutrient Status. <http://www.croplnutrition.com/efu-plant-analysis> (June 16),
61. Campbell, C., Reference sufficiency ranges for plant analysis in the southern region of the United States. **2000**.
62. Hochmuth, G.; Maynard, D.; Vavrina, C.; Hanlon, E.; Simonne, E., Plant tissue analysis and interpretation for vegetable crops in Florida: University of Florida. *Institute of Food and Agricultural Sciences* **2004**, 1-79.
63. McCauley, A.; Jones, C.; Jacobsen, J., Plant nutrient functions and deficiency and

toxicity symptoms. *Nutrient management module* **2009**, 9.

64. Sorrenti, G.; Masiello, C. A.; Toselli, M., Biochar interferes with kiwifruit Fe-nutrition in calcareous soil. *Geoderma* **2016**, 272, 10-19.
65. Chen, X.; Chen, G.; Chen, L.; Chen, Y.; Lehmann, J.; McBride, M. B.; Hay, A. G., Adsorption of copper and zinc by biochars produced from pyrolysis of hardwood and corn straw in aqueous solution. *Bioresource Technol* **2011**, 102, (19), 8877-8884.
66. Hale, S.; Hanley, K.; Lehmann, J.; Zimmerman, A.; Cornelissen, G., Effects of chemical, biological, and physical aging as well as soil addition on the sorption of pyrene to activated carbon and biochar. *Environmental Science & Technology* **2011**, 45, (24), 10445-10453.
67. Runia, W. T., A review of possibilities for disinfection of recirculation water from soilless cultures. *Acta horticulturae: IV International Symposium on Soil and Substrate Infestation and Disinfestation* **1993**, 382, 221-229.
68. Jamart, G.; Prest, G. d.; Kamoen, O., Control of *Pythium* spp. on ornamental plants in a nutrient-film system. *Mededelingen-Faculteit Landbouwkundige en Rijksuniversiteit Gent* **1988**, 53, (2b), 625-634.
69. Garzón, C. D.; Molineros, J. E.; Yáñez, J. M.; Flores, F. J.; del Mar Jiménez-Gasco, M.; Moorman, G. W., Sublethal doses of mefenoxam enhance pythium damping-off of geranium. *Plant Dis.* **2011**, 95, (10), 1233-1238.
70. Dumroese, R.; James, R.; Wenny, D., Trial of a granular etridiazole and thiophanate-methyl mixture to control Fusarium root disease of container-grown Douglas-fir seedlings. *New Forests* **1990**, 4, (3), 231-236.
71. Kuthubutheen, A.; Wickneswari, R.; Kumar Das, V., Efficacy of selected triorganotin (IV) compounds on leaves against *Phytophthora palmivora* (Butler) Butler isolated from black pepper and cocoa. *Applied organometallic chemistry* **1989**, 3, (3), 243-248.
72. Snyman, C.; Kotzé, J., Evaluation of application techniques of four fungicides for the control of *Phytophthora* root rot on avocado seedlings. *S Afr Avocado Growers' Assoc Yrb* **1983**, 6, 79-81.
73. Gubler, F.; Hardham, A.; Duniec, J., Characterising adhesiveness of *Phytophthora cinnamomi* zoospores during encystment. *Protozoa* **1989**, 149, (1), 24-30.
74. Duniway, J. M., Movement of zoospores of *Phytophthora cryptogea* in soils of various textures and matric potentials. *Phytopathology* **1976**, 66, (877-882).
75. Young, B. R.; Newhook, F. J.; Allen, R. N., Motility and chemotactic response of *Phytophthora cinnamomi* zoospores in 'ideal soils'. *Transactions of the British*

Mycological Society **1979**, 72, (3), 395-401.

76. Benjamin, M.; Newhook, F. J., Effect of glass microbeads on *Phytophthora* zoospore motility. *Transactions of the British Mycological Society* **1982**, 78, (1), 43-46.
77. van der Plaats-Niterink, A. J., *Monograph of the genus Pythium*. Centraalbureau voor Schimmelcultures Baarn, Utrecht, The Netherlands: 1981.

CHAPTER 4

CONTROL OF PHYTOPHTHORA CAPSICI DISEASES IN GREENHOUSE SQUASH BY FAST-FLOW FILTRATION

ABSTRACT

Phytophthora spp. are challenging to manage in greenhouse production of ornamentals and vegetables when recirculating irrigation water is used. The objective of this study was to investigate the effectiveness of fast-flow filtration in limiting *Phytophthora* diseases in a model greenhouse vegetable system. Two greenhouse experiments were conducted to test the effect of filter media type (i.e., sand and iron oxide coated media [IOCM]), and application of the fungicide etridiazole in controlling *P. capsici* root and crown rot of squash. Fast-flow filtration through the IOCM filter was more effective in limiting *P. capsici* than the sand filter. The plants in the IOCM filter and fungicide treatments did not display disease symptoms, whereas 44–100% of the plants in the sand filter treatment exhibited disease symptoms including wilting and stunting. Squash plants in the IOCM treatment displayed chlorosis of older leaves and slight stunting symptoms due to nutrient deficiency. Plants treated with fungicide developed phytotoxicity symptoms that included interveinal chlorosis. In summary, plant pathogens such as *Phytophthora* spp. can be removed from irrigation water using fast-flow filtration through the IOCM and this filtration method may be a promising alternative for disease management in vegetable production greenhouses.

4.1. INTRODUCTION

Greenhouses provide optimal conditions to produce vegetables of high quality and yield in a small space. U.S. greenhouse vegetable production increased at a rate of 25% per year from 2002–2006 ¹. Relatively small area of greenhouse production provides a significant amount of production of the total agricultural production value ². Pest management is critical to greenhouse vegetable production ³. The oomycete, *Phytophthora capsici*, is considered one of the most destructive plant pathogens, causing seedling damping-off, stunting, and stem, crown, and root rot in many vegetable crops. This results in limiting production and causing devastating crop losses ⁴⁻⁷. *Phytophthora* and *Pythium* spp. may be introduced to the greenhouse via infected seedling plug plants or soil and dust ^{8, 9}. Once introduced, the pathogen can survive on plant containers, benches, and walkways ¹⁰. Hong and Moorman ⁸ reported that 16 *Phytophthora* spp. and 26 *Pythium* spp. were isolated from nursery and greenhouse operations. Control of *Phytophthora* spp. can be especially challenging because plants can be infected but do not show symptoms until the disease is too advanced for treatment ¹¹.

Recycling irrigation water in the greenhouse offers environmental and economic benefits but presents unique challenges due to the potential of disseminating water mold pathogens such as *Phytophthora* ⁸. Recycling irrigation water in greenhouses is becoming increasingly common. Meador, et al. ¹² reported that 12 of the 24 surveyed greenhouses in the U.S. recycled their irrigation water; the largest daily water use was as high as 1,000,000 gallons. In their study, the three largest water users recycled 75 to 100% of their daily irrigation water. *Phytophthora* spp. release motile zoospores into the flowing water with pathogen transmission exacerbated by irrigation water recycling ^{13, 14}. These

pathogens become established under the warm and wet conditions of the greenhouse environment. *P. capsici*-resistant cultivars are not currently available, and the pathogen's dormant spores (oospores) can survive without a host for many years ¹⁵. More importantly, the cycle of plant infection and sporangia and zoospore production is rapid ^{16, 17}, therefore, even low levels of *Phytophthora* spp. in recycled irrigation water can result in an epidemic if effective water treatment is not implemented ¹⁸.

Various chemical and physical methods have been proposed to disinfect the contaminated recirculation irrigation water in greenhouses, including filtration, chlorination, copper ionization, ozonation, UV light, activated peroxygens, chlorine dioxide, and heat ^{8, 19}. Many of these treatments are costly. Use of effective fungicides is limited due to a lack of products registered for use against *Phytophthora* spp. on vegetables produced in the greenhouse. Development of pathogen resistance to fungicides is also a primary concern with *Phytophthora* spp. ^{16, 20} resulting in a lack of fungicide efficacy and control failure (Lamour and Hausbeck, 2003; Lu et al., 2011). Moreover, phytotoxicity to some crops may occur when high chemical rates are applied ²¹. Water filtration is an cost-effective technique to remove oomycete pathogens such as *Phytophthora* and *Pythium* spp. ^{8, 22, 23}. Currently, screen or disk filters with a pore size > 100 µm are used to remove large particles such as potting soil particles and plant debris to avoid clogging of the irrigation drippers ^{19, 24}. These filters are also used as pre-treatment for other disinfection methods such as heat, ozone or UV radiation treatment ¹⁹. Membrane filters of relatively small pore size (< 5µm, i.e., microfiltration) can be used to remove oomycete pathogens. However, microfiltration often requires relatively high water pressure and costly maintenance due to clogging and leaking problems ²⁵. Slow

sand filtration has been used in drinking water treatment, and also studied for removal of plant pathogens in greenhouse applications^{22, 26-28}. However, sand filtration has rarely been adopted in commercial greenhouses²¹. Slow sand filters are easy to operate and maintain, but water flow is often low at a range of 100–300 L/m²/h^{21, 25}. The low water flow rate can be a limiting factor to the wider adoption of slow sand filtration in the greenhouses. Additionally, the performance of sand filtration for controlling the plant pathogens is variable^{23, 29}. Therefore, more research, particularly on fast-flow filtration, is needed to improve the performance and adoption of filtration for plant pathogen control in the greenhouse.

Removal of *Phytophthora* spp. from irrigation water by depth-bed filter filtration depends on pathogen surface properties (e.g., surface charge, and bio-adhesive) and physicochemical characteristics of filter media such as grain size and surface charge²⁸⁻³¹. A previous study found that most encysted zoospores of *P. capsici* can be removed from recirculating irrigation water, as a result of strong surface attachment, pore straining, and adhesive surface interactions³⁰. Iron-oxide coatings significantly increased the removal of *P. capsici* encysted zoospores³⁰.

The objective of this study was to investigate the effectiveness of fast-flow filtration of recycled irrigation water to limit disease caused by *P. capsici* in a model greenhouse vegetable production system. Six small-scale recirculating irrigation systems simulated ebb-and-flow and flood-floor systems that were constructed to simultaneously test the effect of filter media type (i.e., sand and iron oxide coated media [IOCM]), and fungicide application on controlling *P. capsici*. Squash was used as a model crop because the plant is very susceptible to *P. capsici* and symptoms develop quickly³².

4.2. MATERIALS AND METHODS

4.2.1. Irrigation and Filtration Systems.

Six small-scale irrigation systems were constructed as described³³ and used in this study. Briefly, each irrigation system consisted of an ebb-and-flow bench connected to an optional filtration unit, a 130-L holding tank, and an optional 130-L pre-filter tank. Water flow was delivered by two hydraulic pumps located in the pre-filter and post-filter holding tanks (Figure 4.S1). The system allowed the irrigation water to be pumped into the ebb-and-flow bench, and then be drained back to the holding tank automatically at a predetermined irrigation schedule controlled by timers. When a filter unit was installed, the irrigation water was also passed through the filter unit (Figure 4.S1). The design of the filter unit was described in detail elsewhere³³. The filter was primarily made of capped 6-inch PVC, and packed with either sand (99.69% silica, Granusil®) or granular IOCM. The particle size distribution of the sand was 5.1% of 297-420 μm , 57.2% of 420-595 μm , 36.1% of 595-841 μm , and 1.2 % of ≥ 841 μm . The IOCM was provided by MetaMateria Technologies (Columbus, OH), and manufactured by a proprietary process. A monolith of IOCM was sealed in a fiberglass column. The IOCM is a highly porous ceramic media produced by mixing and curing proper amounts of aluminates, silicates, iron filer, surfactants, and gas forming agents without heating, and then surface-modified with nano-sized iron oxide, resulting a specific surface area of about 100 m^2/g . Its high porosity and interconnected pores contribute to a high water permeability and, subsequently, a fast water flow through the media at a low water pressure. The IOCM was initially developed to sorb phosphorus from water³⁴, and was tested for the removal of *P. capsici* zoospores in this study. The IOCM was provided in the forms of either

granular fragments or a packaged monolith (Figure 4.S2). To prevent clogging, coarse sand (> 500 µm) layers of 2-3 cm were added to both ends of the sand filter and the bottom of the granular IOCM column, but was not added in the monolith IOCM column (Figure 4.S3).

4.2.2. Pathogen Culture and Inoculum.

P. capsici isolate SP98 was obtained from the culture collection of M. K. Hausbeck at Michigan State University. The isolate was originally obtained from pumpkin, is anA2 mating type, and is highly virulent to squash and other vegetable crops⁵. The culturing and production of zoospores were conducted per a previously established method³⁰. Briefly, the *P. capsici* culture was grown on unclarified V-8 agar (UCV8) for 7–8 days. Sterile distilled water was added to the culture and incubated at 4°C for 30–45 minutes, and then at ambient temperature for 30 minutes so as to prompt the release of zoospores from sporangia. The prepared zoospore suspension was transferred into a 2-L Erlenmeyer flask. To determine the concentration of the zoospore suspension, a 1-mL aliquot was placed into a 1.7-mL microcentrifuge tube, and vortexed for 70 seconds to induce zoospore encystment. Afterwards, a 10-µL aliquot of the encysted zoospore suspension was pipetted onto a clean hemacytometer for enumeration (Bright-Line, Hausser Scientific, Horsham PA). The concentration of the zoospore suspension was adjusted to 4×10^5 zoospores/mL. The prepared suspension of motile biflagellate zoospores was equally split into 500-mL capped bottles, and hand-shaken vigorously for 90 seconds to induce the zoospore encystment. The encysted zoospore suspension was used to infest the irrigation water in the following greenhouse experiments.

4.2.3. Greenhouse Experiments.

Two greenhouse experiments were conducted to evaluate the effectiveness of filtration to limit *P. capsici* transmission. The two experiments A and B differed in duration and the type of IOCM used, lasting for 11 and 19 days, respectively. The granular IOCM was used in experiment A, and the monolith IOCM in experiment B. Experiment A included 6 treatments, including: 1) non-inoculated control without filter (–Control); 2) inoculated control without filter (+Control); 3) non-inoculated with sand filter; 4) non-inoculated with the granular IOCM filter; 5) inoculated with the sand filter; 6) inoculated with the granular IOCM filter. The 3rd and 4th treatments were included to evaluate if the squash growth would be affected by the presence of the filter media. Experiment B had 5 treatments, including: 1) non-inoculated control without filter (–Control); 2) inoculated control without filter (+Control); 3) inoculated with the sand filter; 4) inoculated with the IOCM monolith filter; 5) inoculated with the fungicide etridiazole (Terrazole 35 WP; OHP, Mainland, PA) applied at the labeled rate (250 mg/L) into the irrigation water in the holding tank prior to the initial inoculation. No filter was used in the fungicide treatment, and fungicide was not applied to the other treatments.

Twelve-day-old seedlings of acorn squash ‘Table Ace’ (*Cucurbita pepo*) were grown in 5-inch greenhouse pots with a peat potting mix (Suremix, SunGro, Galesburg, MI) were used in the experiments. To ensure a sufficient number of replicates, 8–9 plants were placed on each of the ebb-and-flow benches (Hummert, St. Louis, MO) in experiment A, and 15 plants in experiment B. A 20-20-20 water-soluble fertilizer (Scotts Company, OH) was added to the irrigation water, and the initial nutrient concentration was 100 mg/L based on nitrogen. Groundwater was used as the source of irrigation water

and had a pH of 7.7 ± 0.4 and electrical conductivity (EC) of $854 \pm 84 \mu\text{S}$. The pre-filter tank in the filter treatments or the holding tank in the filter-free treatments was filled with 120 L of the fertilized irrigation water. Then, 500 mL of the prepared suspension of encysted zoospores was added and agitated with a wooden dowel, resulting in a concentration of 1.7×10^3 zoospores/mL. During the filtration, the irrigation water was passed through the filter unit and then stored in the holding tank before the next irrigation. The squash plants were irrigated for 10 minutes, twice each day to prevent oversaturation of the growing media in the pots. To maintain a sufficient amount of water during each experiment, the fertilized irrigation water was added when the water volume decreased to about 70–80% of the initial volume. Water flow velocity and residence time were calculated based on 3-day averages at the beginning of each experiment ($n = 6$). During the experiments, the irrigation water was sampled from the holding tanks periodically to determine pH, EC, nutrient levels, and zoospore concentrations. The pH and EC of the irrigation water were measured to be 7.8 ± 0.5 and $1.1 \pm 0.2 \text{ mS}$, respectively, during the experimental periods. The water pressure inside the filter unit was monitored in real time, along with the water temperature in the holding tank, and air temperature and relative humidity in the greenhouse. The air temperature and relative humidity were $28.9 \pm 2.2 \text{ }^\circ\text{C}$ and $41.9 \pm 8.6 \%$, respectively, in experiment A, and $25.7 \pm 3.1 \text{ }^\circ\text{C}$ and $16.6 \pm 6.2 \%$, respectively, in experiment B (Figure 4.S4).

4.2.4. Filtration Performance Assessments

To evaluate the performance of the filtration systems in controlling *P. capsici* outbreaks in squash, the plants were evaluated for foliar and root biomass at the end of each experiment. At harvest, the roots were carefully washed, and fresh biomass of the

squash shoots and roots were measured. The shoot and root samples were then oven-dried at 60°C for 3 days to determine dry biomass determined. In experiment A, the root morphology was imaged and examined, and the zoospore concentration in the holding tank was measured. Briefly, a 50 ml aliquot of water was collected from each tank immediately after agitation, and returned to the laboratory. The suspension was immediately filtered through 2.5 µm pore-size quantitative filter paper (GE Healthcare, Pittsburg, PA), and the filter paper plated onto BARP (50 mg L⁻¹ benomyl, 100 mg L⁻¹ ampicillin, 30 mg L⁻¹ rifampicin, 200 mg L⁻¹ pentachloronitrobenzene)-amended CV8 (i.e., 100 mL V8 juice filtered through 4 layers of cheeses cloth, 3 g CaCO₃, 16 g agar L⁻¹). After 2 days, the filter paper was removed and colonies were enumerated. In experiment B, the percentange of wilted plants was determined throughout the study to illustrate infection progress.

4.2.5. Chemical Analyses

To assess if the IOCM released dissolved iron or iron oxide particles during the experiments, 120 mL water samples were collected from the holding tank at the begging, middle, and end of experiments. The collected samples were divided into two subsamples. One set of subsamples were filtered through the 0.45-µm membrane filter using a vacuum pump. Then 10 mL of each non-filtered or filtered water sample was added into a 50-mL digestion tube, followed by the addition of 4 N HCl. The digestion tubes were heated at 90°C for 3 hours using a digestion block (Magnum Series; Martin Machine, IL) to completely dissolve iron oxides. Afterwards, the tube was filled with DI water to 25 mL. The concentration of iron in the digested water samples was measured by atomic absorption spectroscopy (AAnalyst 400, PerkinElmer, Waltham, MA).

To measure the ζ -potentials of IOCM, 1 gram was gently ground using a pestle and mortar. The ground IOCM was ultrasonicated in 10 mL DI water for 10 minutes and settled for 30 minutes. Then 1 mL of the supernatant was taken for the ζ -potential measurements using a Malvern ZetaSizer Nano ZS (Malvern, Westborough, MA).

4.2.6. Statistical Analysis

Statistical analyses on the experimental data were performed with the R software using the “LSD” R package for parametric test. Comparison among treatments were made by one-way analysis of variance ($p \leq 0.05$). When a significant F value was determined, means were separated by the LSD’s multiple comparison test.

4.3. RESULTS

4.3.1. Irrigation and Filtration Systems

The water flow velocities for the IOCM and sand filters were 23.7 ± 3.9 and 5.5 ± 1.62 cm/min in experiment A, and 22.0 ± 2.3 and 6.7 ± 3.3 cm/min in experiment B. Consequently, the water residence time for the IOCM and sand filters were 2.2 ± 0.4 and 10.2 ± 3.6 minutes in experiment A, and 2.2 ± 2.32 and 9.4 ± 4.5 minutes in experiment B. Operating water pressure of the IOCM and sand filters was maintained at 10.5 ± 6.6 kPa (i.e., 1.5 ± 0.96 psi) and 9.1 ± 1.9 kPa (i.e., 1.3 ± 0.28 psi), respectively, under typical greenhouse water temperatures (Figure 4.S5 and 4.S6). Because rapid sand filtration often has a water velocity of 8.3–25 cm/min³⁵⁻³⁷, the IOCM filter allows a fast flow rate. The water velocity in the sand filter was at least 13 times of that in typical slow sand filtration (0.17–0.5 cm/min)³⁸. For this research, a high water flow was established under low water pressure provides irrigation efficiency that can be advantageous to

greenhouses that need to deliver high water volume in a short time while conserving energy use.

In terms of irrigation water quality, the concentration of NO_3^- , P, and Mg were lower, and the concentration of K and Na were higher in the IOCM filter effluent, compared with other treatments in experiment B (Table 4.S1). The isoelectric point of iron oxide typically ranges from 7.5 to 9.0^{39, 40} and the ζ -potential is 13.1 ± 1 mV at pH 7.6. Therefore, the IOCM surface might be protonated and become positively charged at the experimental pH of 7.8 ± 0.5 , thus providing sorption sites for the negatively charged NO_3^- and phosphate ions. Additionally, the negatively charged surface sites in the aluminosilicate material in the IOCM could also bind cation such as Mg^{2+} , thus decreasing its concentration. The increased K and Na concentrations likely resulted from the release of these ions from the IOCM (Table 4.S1). Moreover, iron oxide particles could be released from the granular IOCM in experiment A. The iron concentrations in the non-filtered and filtered water samples were as high as 7 and 1.96 mg/L, respectively (Table 4.S2). In contrast, the iron concentrations in the non-filtered and filter water samples were 2.39 mg/L, at the maximum, and non-detectable in the monolith IOCM, respectively (Table 4.S3). While the IOCM appeared to release iron, the iron levels in the irrigation water would not cause plant phytotoxicity^{41, 42}. Also, this release may decrease with time as less strongly attached iron nano particles will be washed off with repeat washing.

The concentration of *P. capsici* in the irrigation water determined by colony forming unit (CFU). The zoospore concentration in the irrigation water for the inoculated sand and IOCM filter treatments was decreased by more than 90% from the concentration

in the inoculated control treatment (Table 4.S4), consistent previous studies ³⁰. The zoospore concentration remained low for the duration of the experiment. At the final zoospore measurement, the irrigation water in the sand filter treatment contained 83% fewer zoospores than the inoculated control treatment, whereas the irrigation water in the IOCM filter treatment had no zoospores.

4.3.2. Plant Assessment

In the inoculated control treatment, significant plant damage was observed at the end of experiment A (Figure 4.1), and the squash plants were dead and dried at the end of experiment B (Figure 4.2), due to a longer exposure to *P. capsici*. A visual change was not observed among the squash plants in the non-inoculated sand filter and IOCM filter treatments, compared with those in the non-inoculated control treatment. Thus, the filter media did not negatively affect plant growth. In both experiments, 44–100% of the plants in the sand filter treatment appeared to be infected by *P. capsici*, and exhibited wilting and stunting (Figure 4.1 and 4.2). The plants in the IOCM filter treatments did not display wilt symptom. The plants in the treatment that included etridiazole application to the irrigation water in experiment B showed phytotoxicity symptoms including interveinal chlorosis (Figure 4.S7). The visual appearance of the squash shoots were in accordance with their root morphology (Figure 4.S8). In experiment A, the roots of all the plants in the inoculated control treatment and 44% of the plant roots in the sand filter treatment displayed root rots, whereas the plant roots in other treatments were healthy. The wilt symptom of the plants was monitored throughout experiment B (Table 4.1). In the inoculated control treatment, all of plants wilted and died 6 days after inoculation. Disease development was slower in the sand filter treatment, and irreversible wilting was

observed 19 d after inoculation. No wilt symptoms were observed for plants in the other treatments. Sand filtration slowed but did not completely prevent disease development in the squash plants. The IOCM filter and fungicide treatments effectively prevented disease symptoms.

The visual observations of the plants were in agreement with the plant biomass measurements. In the absence of *P. capsici*, the weights of fresh and dry leaves and roots in the sand and IOCM filter treatments were not significantly different from that in the non-inoculated control treatment, confirming that the two filter media did not negatively affect the squash growth. In experiment A, the fresh and dry weights of squash leaves and roots in the sand and IOCM filter treatments were similar to those of plants in the non-inoculated control treatment. The plants in the inoculated sand filter treatment had a significantly lower foliar fresh weight (Figure 4.3). While the plants in the inoculated sand filter treatment visually appeared inferior to those in the inoculated IOCM filter treatment, the measured biomass was not significantly different. In experiment B, the plant biomass in the inoculated IOCM filter treatment was significantly higher ($p \leq 0.05$) than that of the plants in the inoculated control and sand filter treatments. The foliar fresh weight of the plants in the inoculated sand filter treatment was, on average, 72% less than that of the inoculated IOCM filter treatment. There was no significant difference in the plant biomass between the fungicide treatment and the sand and IOCM filter treatments. The non-inoculated control treatment had significantly higher plant biomass than all other treatments (Figure 4.4).

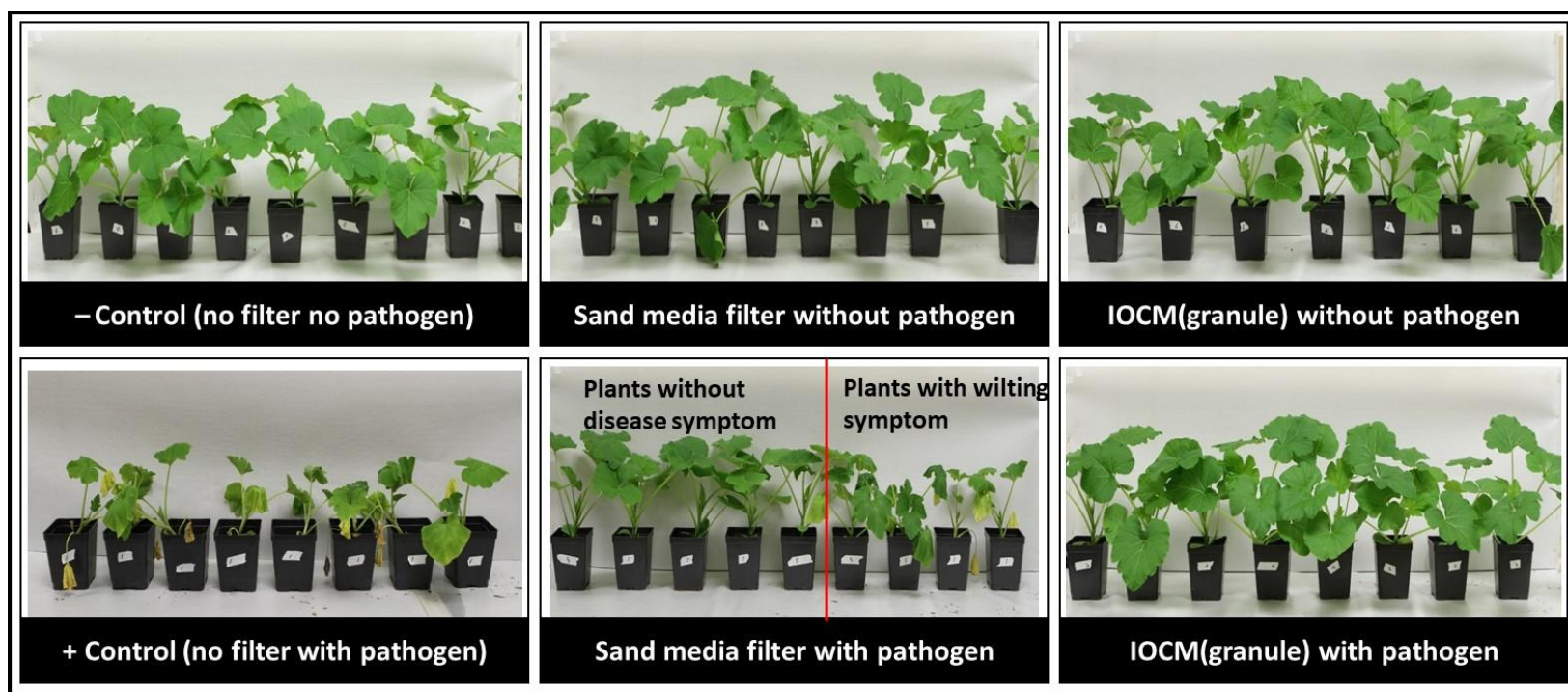


Figure 4.1. Images of squash plants at the end of the Experiment A (11 days after inoculation).

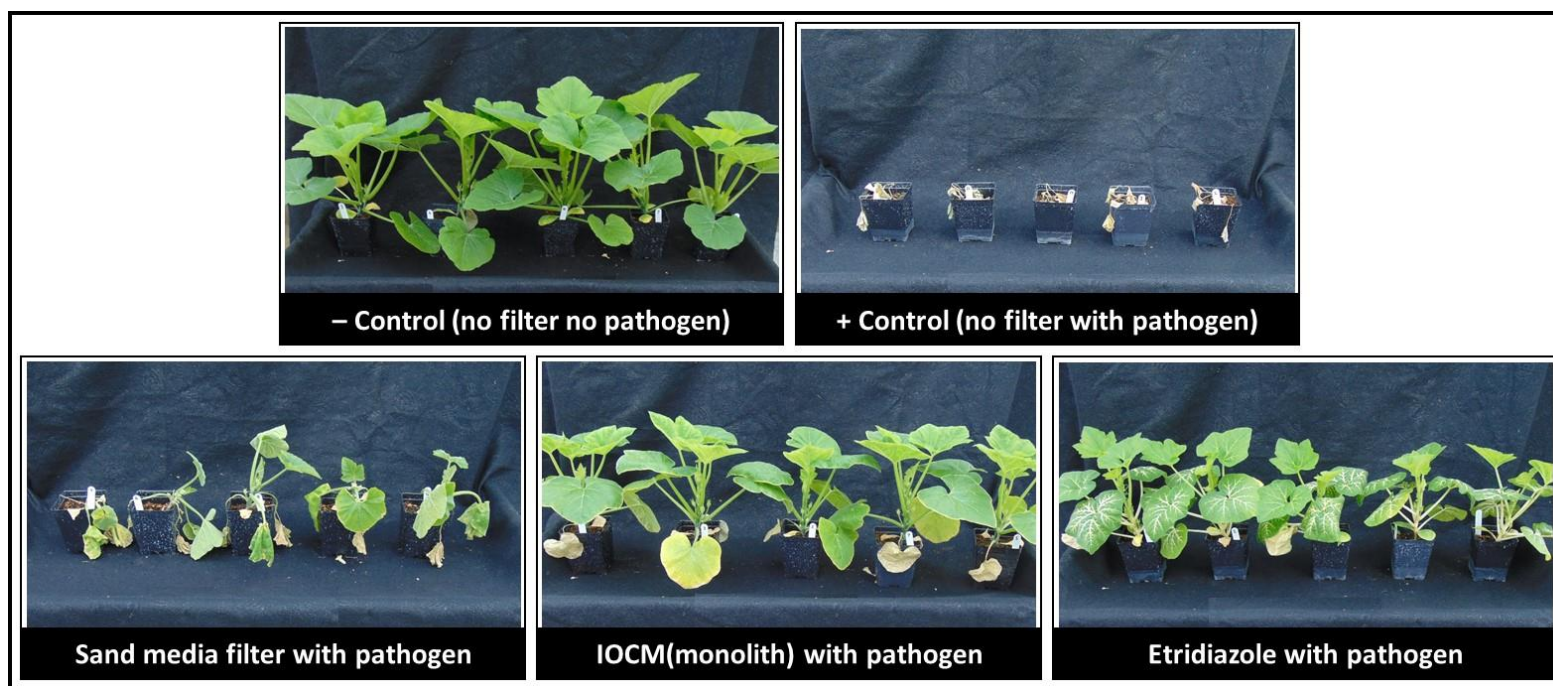


Figure 4.2. Representative images of squash plants at the end of the Experiment B (19 days after inoculation).

Table 4.1. Percentage of the plants with wilting symptoms in the Experiment B.

Treatment	Days after inoculation											
	4	5	6	10	11	12	13	14	15	16	17	19
–Control	0	0	0	0	0	0	0	0	0	0	0	0
+Control	0	13	100	100	100	100	100	100	100	100	100	100
Sand filter	0	0	0	13	33	40	40	67	80	93	93	100
IOCM filter	0	0	0	0	0	0	0	0	0	0	0	0
Terrazole 35 WP	0	0	0	0	0	0	0	0	0	0	0	0

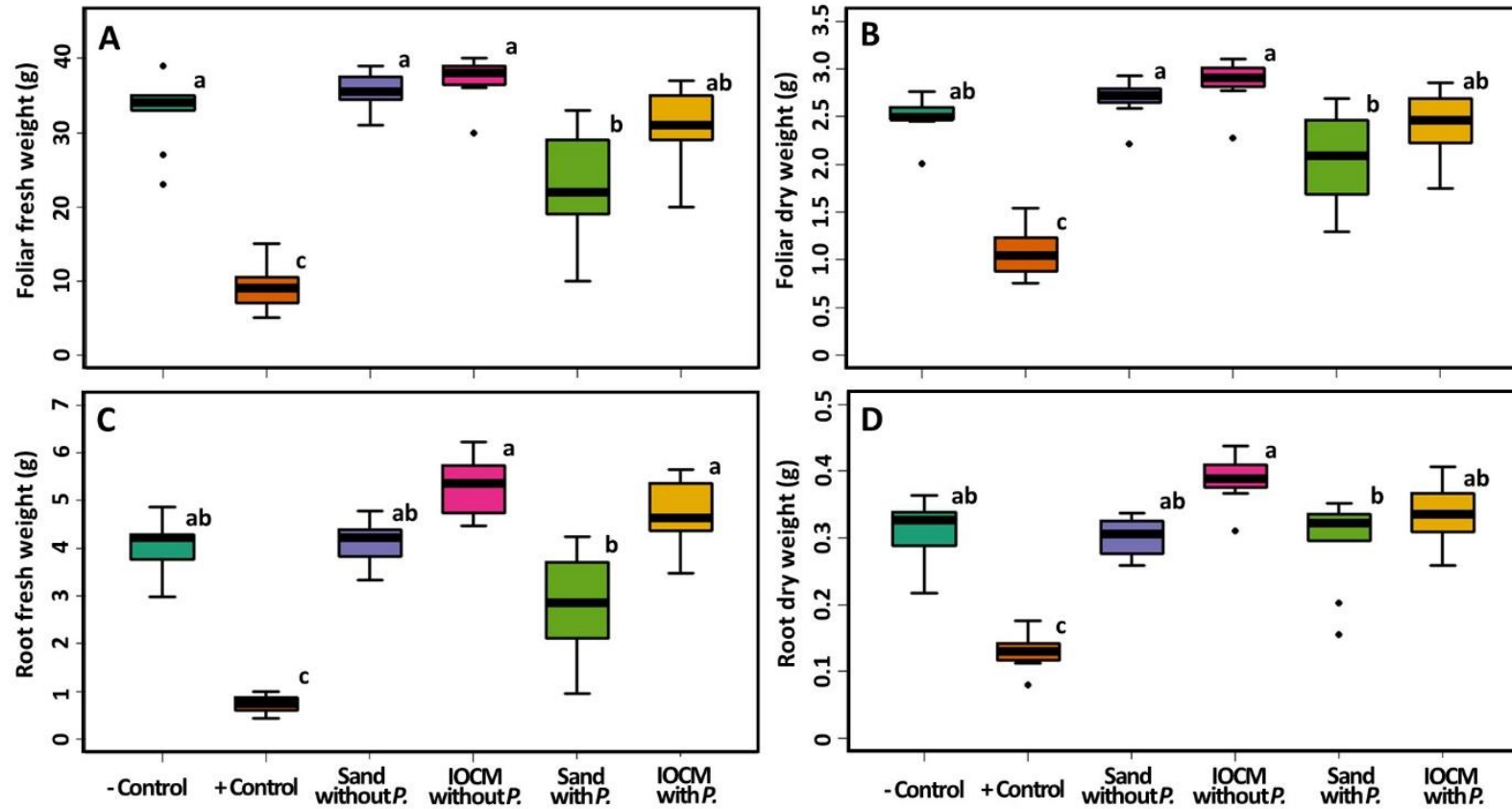


Figure 4.3. Squash foliar fresh (A) and dry (B) weight, and root fresh (C) and dry (D) weight in the Experiment A (LSD test, $P < 0.05$).

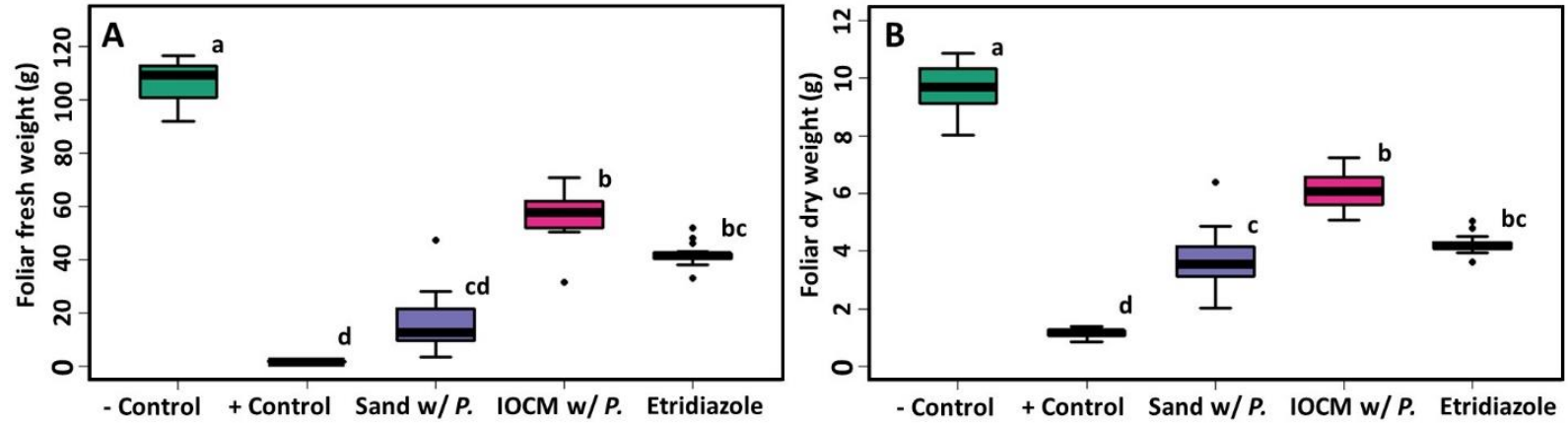


Figure 4.4. Squash foliar fresh (A) and dry (B) weight in the Experiment B (LSD test, $P < 0.05$).

4.4. DISCUSSION

4.4.1. Filtration Performance

This results showed that the squash plants irrigated with water after fast-flow IOCM filtration were of higher quality and biomass compared to plants in the sand filtration treatment. This likely resulted from the increased removal of *P. capsici* zoospores in the IOCM filter than in the sand filter. The improved disease control observed in the IOCM filter treatment could have resulted from greater electrostatic attraction of the negatively charged zoospores to the positively charged iron oxide surface sites in the IOCM, compared to the negatively charged sand surface ^{30, 40, 43, 44}. The biomass decrease in squash in the IOCM treatment probably resulted from nutrient removal by the IOCM (Table 4.S1). The squash plants in the IOCM treatment displayed chlorosis of older leaves and slight stunting symptoms. These are common symptoms of nitrogen, phosphorus, and magnesium deficiency ⁴⁵. Thus, application of supplemental nutrient in irrigation water or slow release fertilizer in pots would be needed when the IOCM filter is used in filtering recycled nutrient solutions.

Sand filter media effectively limited *P. capsici* zoospores, but did not completely prevent disease. Os et.al (1998) reported that physical factors such as pore straining is an important mechanism in the removal of pythiaceus zoospores rather than surface charge. Many previous studies conducted in laboratories and greenhouses have consistently shown that sand filters can effectively remove pythiaceus zoospores from water ^{26-30, 46, 47}. However, in this study 100 % of the squash plants died 19 days after the initial inoculation. It appears that despite the substantial removal of *P. capsici* zoospores, a small percentage of the zoospores may have passed through the sand filter due to the

initial high pathogen inoculum concentration (1.7×10^4 zoospores/mL) ³⁰. Indeed, it was reported that *Pythium* sp. and *Phytophthora* sp. were efficiently removed when the water flow was less than 0.5 cm/min and the pathogen loading was not high ³⁸. Jeon et. al. (2017) confirmed that *Pythium* zoospores were effectively removed in the fast sand filter at a low pathogen inoculum concentration of 68 zoospores/mL. The isolate of *P. capsici* that was used is highly virulent and the squash used in the trial is highly susceptible ⁴⁸, thus even a low concentration of transported zoospores could cause serious crop damage ^{29, 49}. Also, there was not time in this study for the rapid sand filters to develop a biofilm (or Schmutzdecke) as each was conditioned with irrigation water one or two days before inoculation. The active biofilm layer on the sand surface was considered the most important factor for a high pathogen removal efficiency ⁵⁰. Lee and Oki ²⁶ reported that it took more ten days to reach a 100% reduction of *P. capsici* using the slow sand filter. However, others suggested that physicochemical factors (e.g., grain size, grain surface properties, and solution chemistry) are more important in controlling the removal of phythiaceous zoospores ²⁸⁻³¹.

4.4.2. Fungicide Application

The fungicide treatment provided a comparison among the filtration treatments in experiment B. The plant biomass from the fungicide treatment was not different from that of the sand and IOCM treatments, but was significantly less than the non-inoculated control. While wilting was not observed, the plant growth in the fungicide treatment was negatively impacted, possibly due to fungicide phytotoxicity. Significant chlorosis was observed on the foliage (Figure 4.S7). Other studies have indicated that etridiazole can cause phytotoxicity symptoms in avocado seedlings, Douglas-fir seedlings, and black

pepper leaves⁵¹⁻⁵³. Etridiazole is not registered for food crops but is commonly used in the greenhouse, especially for control of *Pythium* spp. The fungicides mefenoxam (active enantiomer of metalaxyl; Subdue Maxx; Syngenta Crop Protection, Greensborough, NC) and etridiazole have historically been used to manage root rot of ornamentals in greenhouse production⁵⁴⁻⁵⁶ and provide effective control when applied as soil-drenches⁵⁶⁻⁵⁸. Resistance to mefenoxam has developed in greenhouse populations of *Pythium* and *Phytophthora* due to repeated fungicide use^{59, 60 55, 61}. Greenhouses that recycle irrigation water face additional challenges because sub-lethal levels of recycled fungicides can exert selection pressure on pathogen populations to develop fungicide resistance^{61, 62}. Overall, in addition to the observed phytotoxicity, the fungicide application management option is not ideal because: the availability of effective fungicides in the market is limited; *Phytophthora* spp. often quickly develop fungicide resistance; and the fungicide discharge in wastewater raises environmental concerns^{16, 20}. Finally, the etridiazole treatment may not be as effective against *P. capsici* as recently registered systemic fungicides⁶³. However, many of these fungicides are not labeled for greenhouse vegetable production. Thus, it is critical to understand the target pathogen, the pathogen loading, fungicide efficacy, and plant susceptibility in order to design an effective sand filtration system and integrated disease management program.

4.5. IMPLICATIONS

Contamination of recirculating irrigation water by oomycete pathogens is a major threat to greenhouse producers of vegetables and floriculture crops. Many greenhouse operations recycle irrigation water due to environmental regulation and public concern

regarding water and pesticide use. Control of oomycete pathogens in recirculating irrigation water is critical to the greenhouse industry. This study indicates that physical removal of pathogens using fast-flow may be a promising tool for disease management in the greenhouses. Previous studies using the fast-flow filtration to remove plant pathogens from irrigation water have been limited. This study found that the IOCM filter could effectively protect squash plants from *P. capsici*, but resulted in nutrient deficiency. The sand filter did not prevent, but slowed the disease in the squash. Thus, it is clear that the IOCM filter has the potential to be used in treating irrigation water in greenhouse vegetable production, but nutrients will need to be supplied separately to the recirculating irrigation water to avoid nutrient deficiency. Fast-flow filtration has the potential for greenhouse disease management with future work needed to develop improved filtration systems (i.e., filter media type, operation parameters, and process design) that limit plant pathogens for an extended period while maintaining sufficient nutrient levels for plant growth.

4.6. ACKNOWLEDGMENTS

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recommendation for use.

APPENDIX

APPENDIX

SUPPORTING INFORMATION

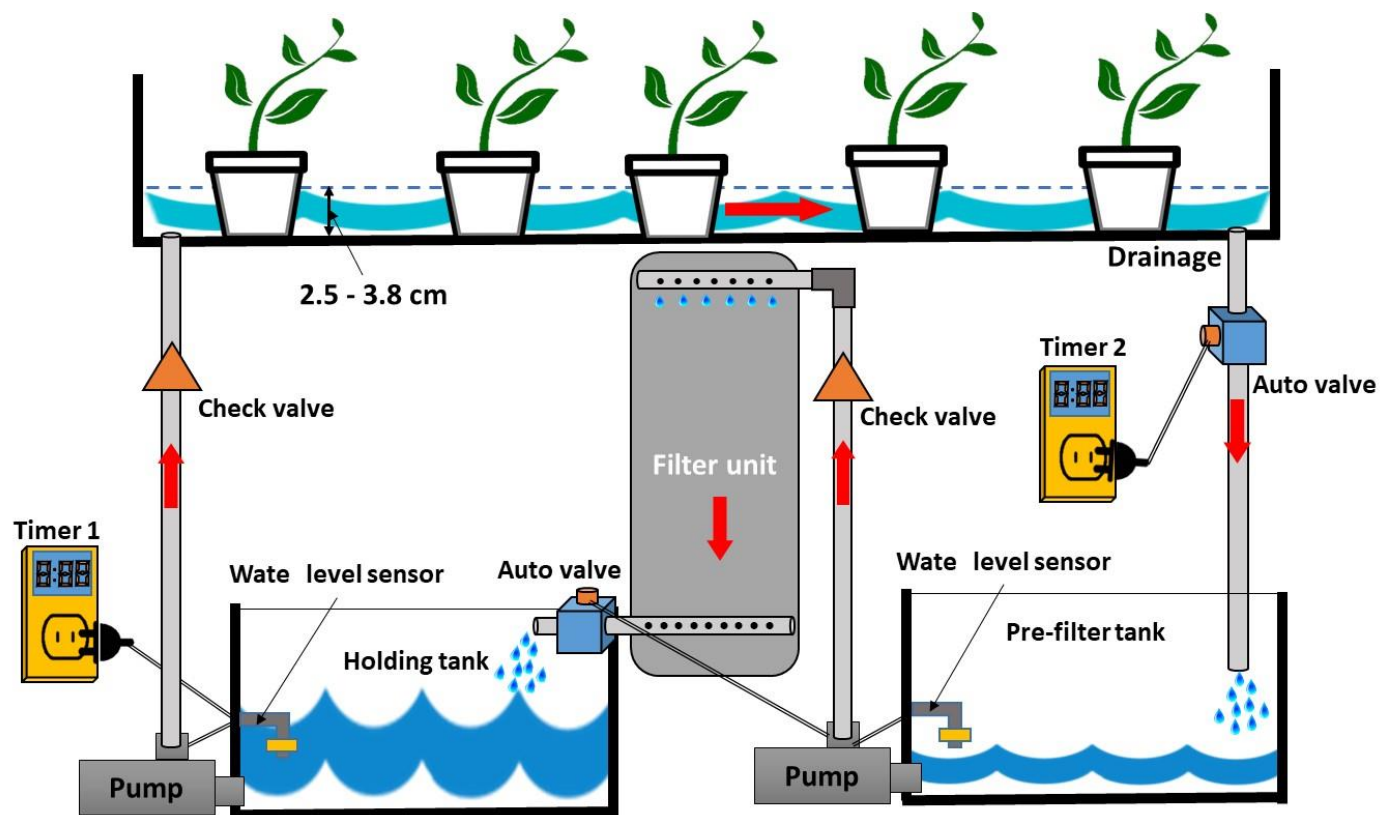


Figure 4.S1. Scheme of the ebb-and-flow irrigation system constructed in the greenhouse.

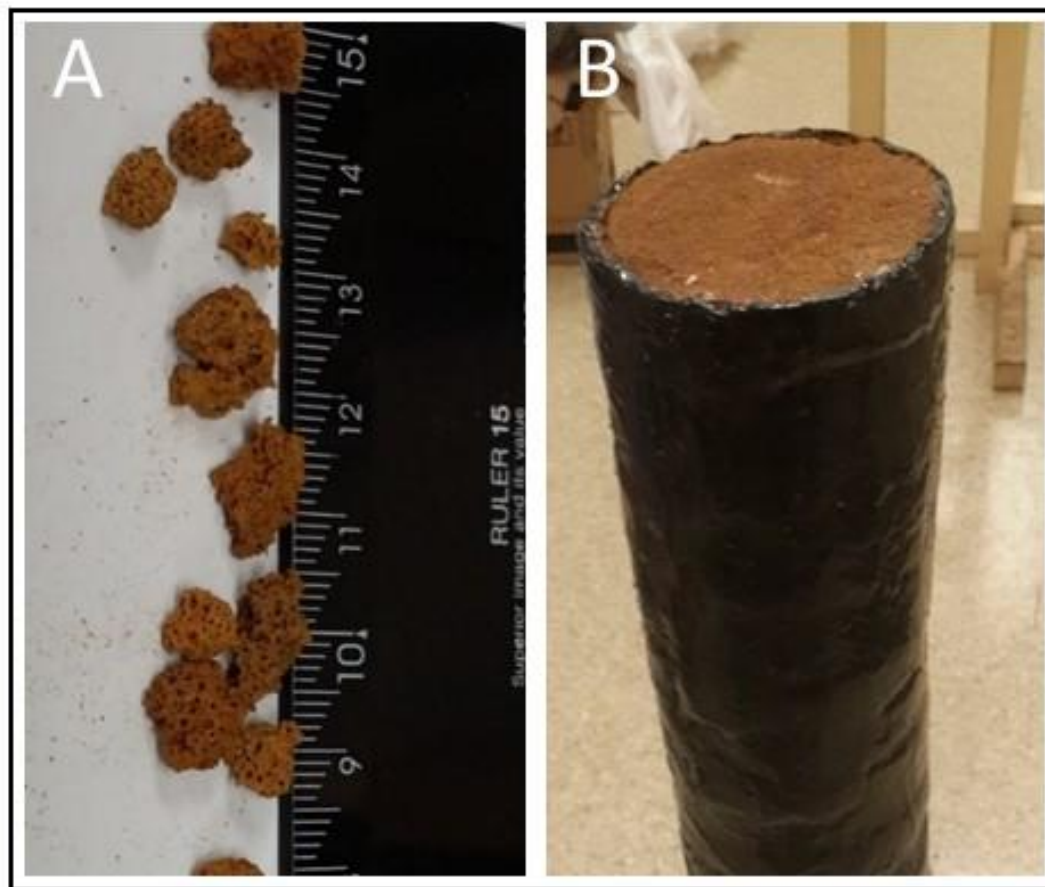


Figure 4.S2. Granular (A) and monolith (B) iron oxide coated media (IOCM).

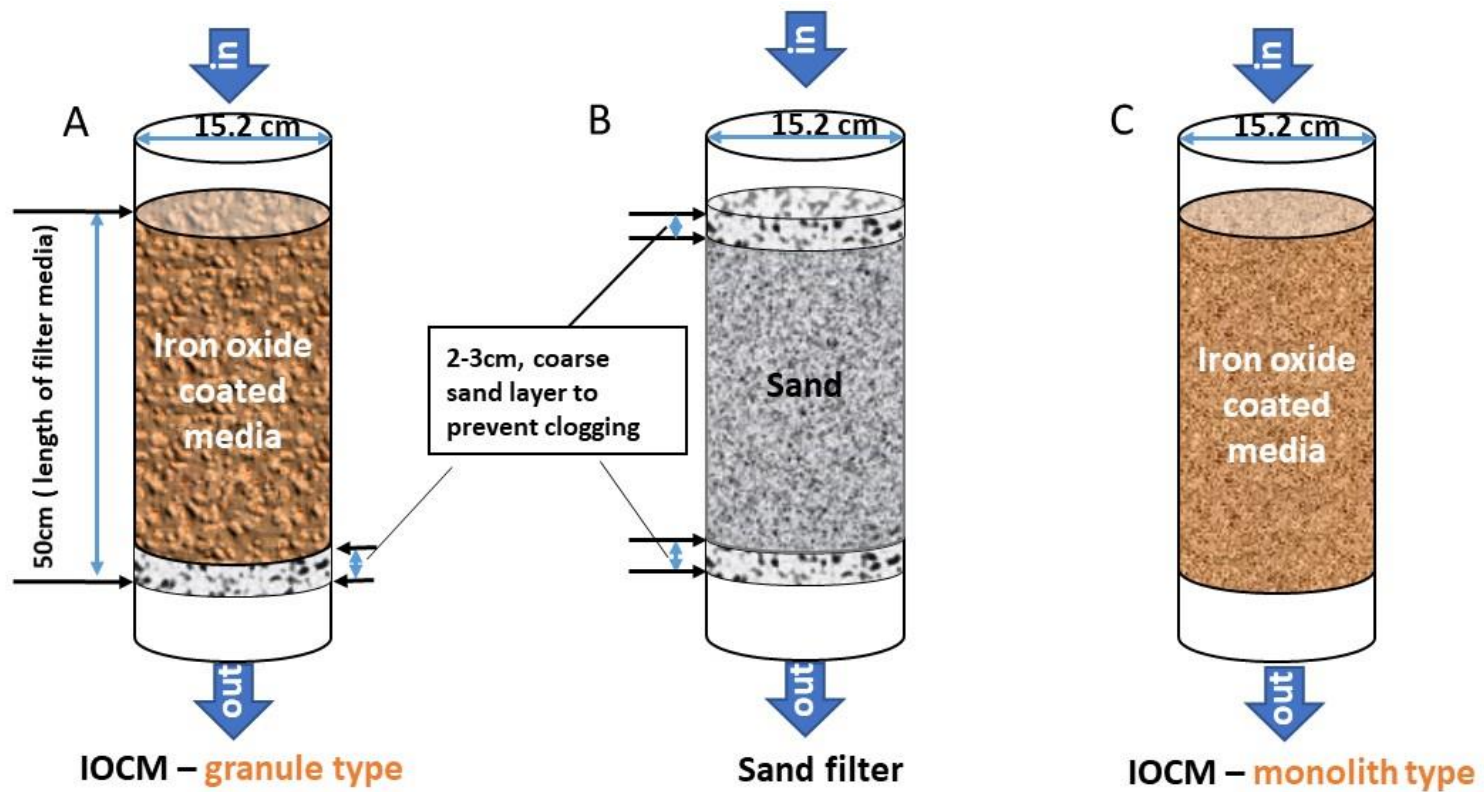


Figure 4.S3. Filter design of granular IOCM (A), sand (B), and monolith IOCM (C).

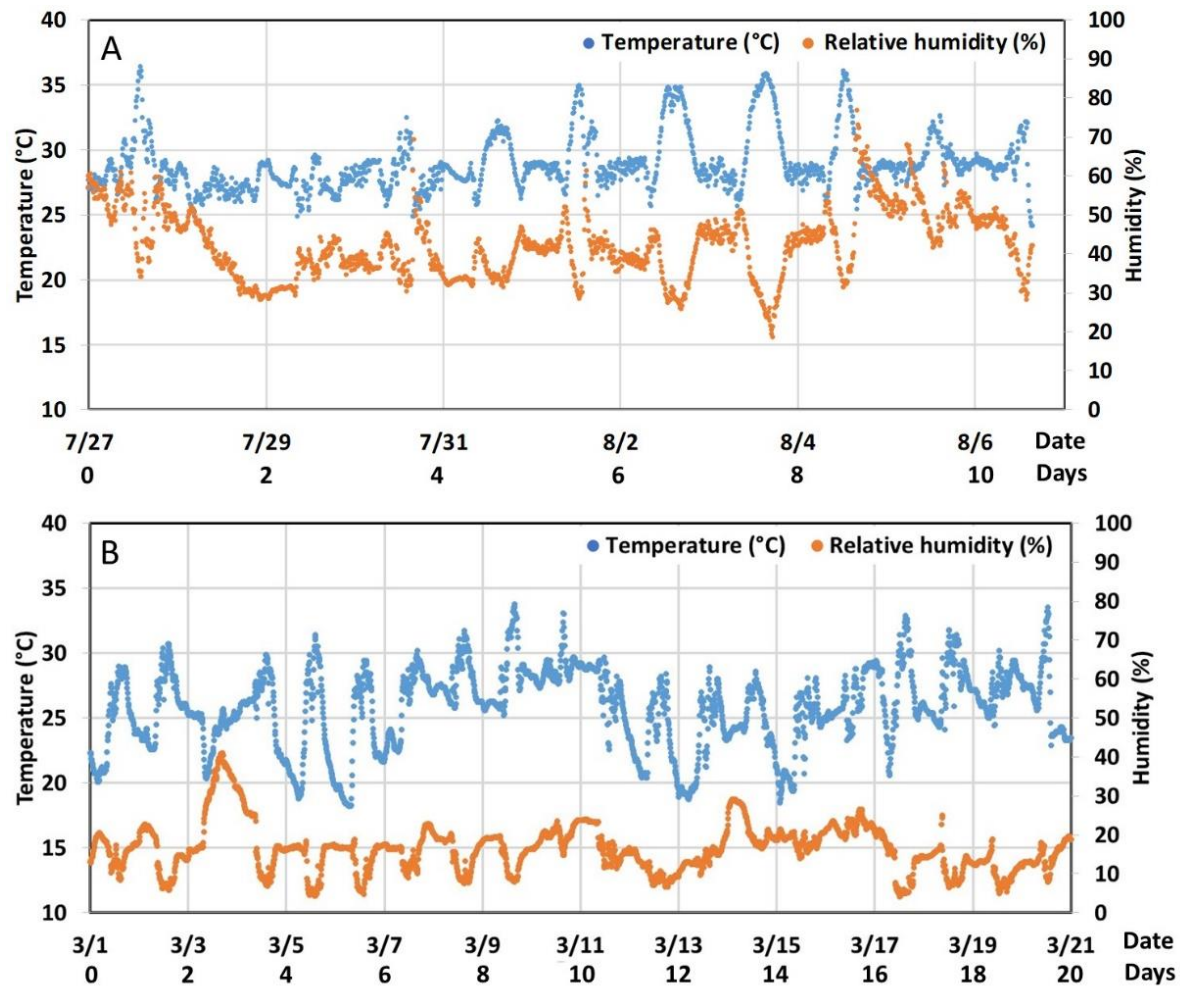


Figure 4.S4. Air temperature and relative humidity during the Experiment A (A) and Experiment B (B) in the greenhouse.

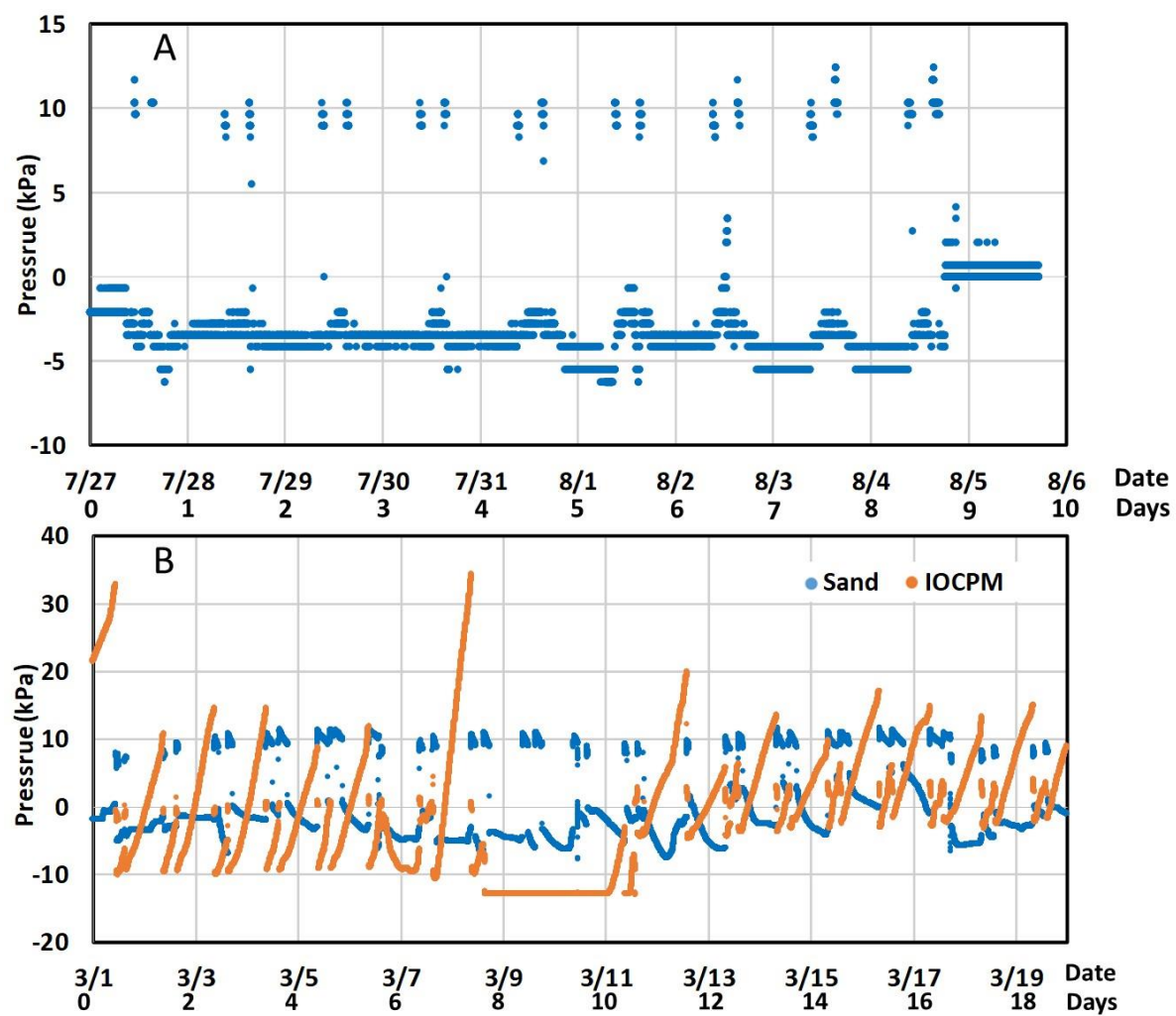


Figure 4.S5. Water pressure in the sand filter in the Experiment A (A) and in the sand and IOCPM filters in the Experiment B (B).

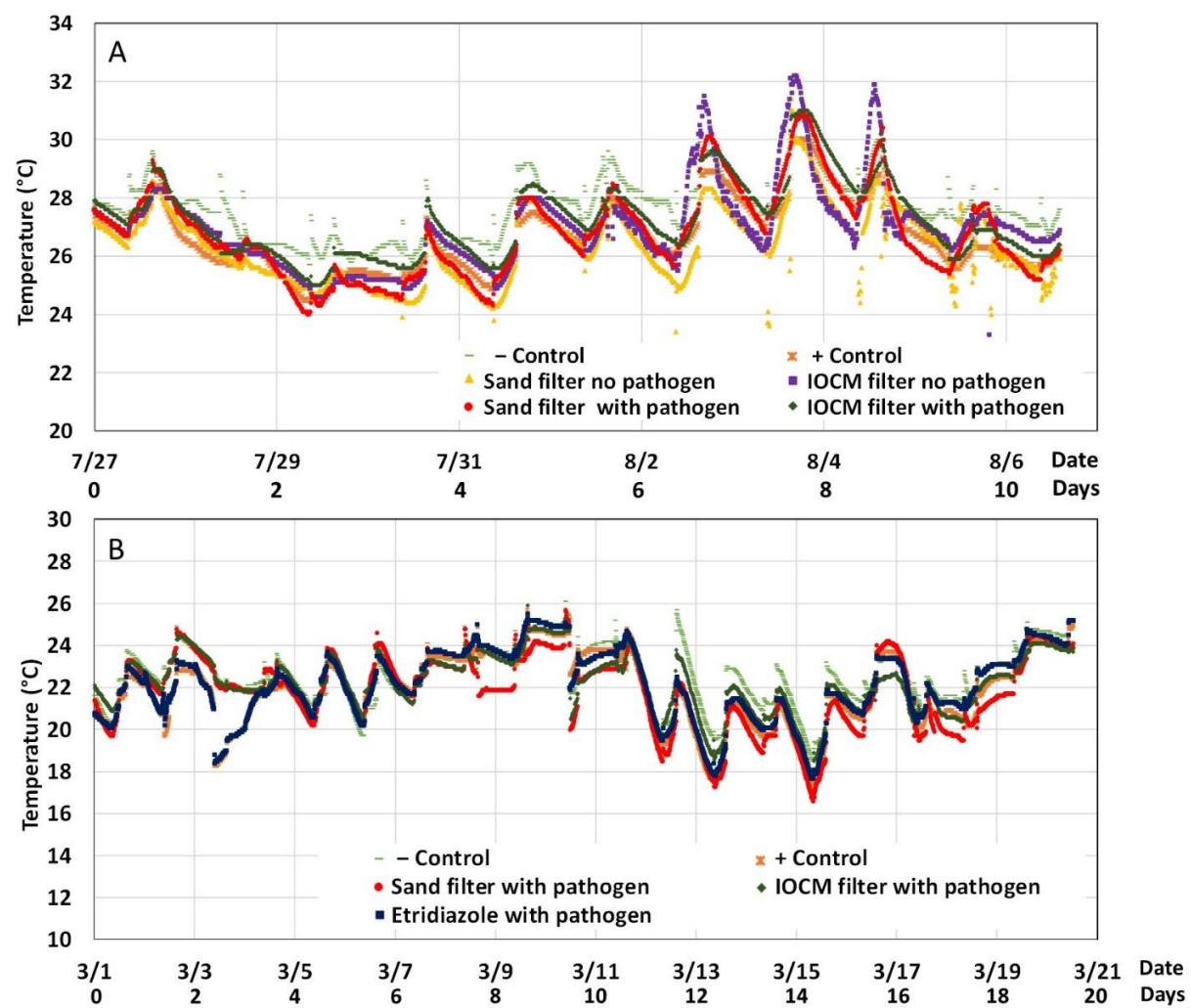


Figure 4.S6. Water temperature in the holding tank during the Experiment A (A) and Experiment B (B).

Table 4.S1. Nutrient concentrations in irrigation water during the Experiment B.

Treatments	mg/L																	
	NO ₃ ⁻			P			K			Ca			Mg			Na		
	Days ^a	3	10	19	3	10	19	3	10	19	3	10	19	3	10	19	3	10
- Control	29	30	51	24	17.6	21.1	93	89	110	41	55	68	38	35	41	18	20	22
+ Control	16	32	56	12.8	16	17.6	53	84	120	27	41	55	30	29	36	11	16	23
Sand filter	27	24	35	19	13.2	11	84	72	80	55	55	68	33	30	36	18	16	19
IOCPM filter	17	1.9	0.4	0.1	2.1	0.1	123	111	137	68	68	55	17	19	19	54	46	52
Etridiazole	28	24	27	12	12.8	6.6	89	78	87	55	41	41	30	29	35	24	19	23
Ground water	0	ND	ND	0.1	ND	ND	2	ND	ND	123	ND	ND	35	ND	ND	18	ND	ND

^a The numbers in the “days” means the days from the start of the experiment (i.e., the inoculation).

^b ND means “no data available”

Table 4.S2. Iron concentrations in the filtered and non-filtered irrigation water in the Experiment A.

	5 days (August 1) ^a		11 days (August 7)	
	Filtered	Non-filtered	Filtered	Non-filtered
	mg/L			
–Control	NA ^b	0.19	ND ^c	0.50
Sand filter no <i>P.</i>	NA	0.31	0.16	0.02
IOCM filter no <i>P.</i>	NA	0.63	0.21	0.47
No filter with <i>P.</i>	NA	0.44	0.04	0.34
Sand filter with <i>P.</i>	NA	0.32	LOD	0.08
IOCPM filter with <i>P.</i>	NA	7.00	1.96	6.43

^a Days means the days after the inoculation, and the sampling date in 2014 is provided in the parentheses.

^b NA means “no data available” due to missing of filtration procedure.

^c ND means the concentrations below the limit of detection.

Table 4.S3. Iron concentrations in the filtered and non-filtered irrigation water in the Experiment B

Exp. A	3 days (March 4) ^a		10 days (March 11)		19 days (March 20)	
	Filtered	Non-filtered	Filtered	Non-filtered	Filtered	Non-filtered
- Control	ND ^b	ND	ND	ND	ND	ND
+ Control	ND	ND	ND	ND	ND	ND
Sand filter	ND	0.02	ND	ND	ND	ND
IOCPM filter	ND	0.61	ND	2.39	ND	0.42
Terrazole	ND	ND	ND	ND	ND	ND

^a Days means the days after the inoculation, and the sampling date in 2015 is provided in the parentheses.

^b ND means the concentration below the limit of detection.

Table 4.S4. Zoospore concentrations in the Experiment A by colony forming units ^a.

Treatments	Days (Sampling Date) ^b		
	0 (July 27)	2 (July 29)	6 (August 2)
- Control	0	0	0
Sand filter no <i>P</i> .	0	0	0
IOCM filter no <i>P</i> .	0	0	0
No filter with <i>P</i> .	6.7	20.4	2.4
Sand filter with <i>P</i> .	6.1	0.2	0
IOCPM filter with <i>P</i> .	4.1	0.4	0.4

^a Colony forming units (CFU) = CFU/mL

^b Days means the days after the inoculation, and the sampling date in 2014 is provided in the parentheses.

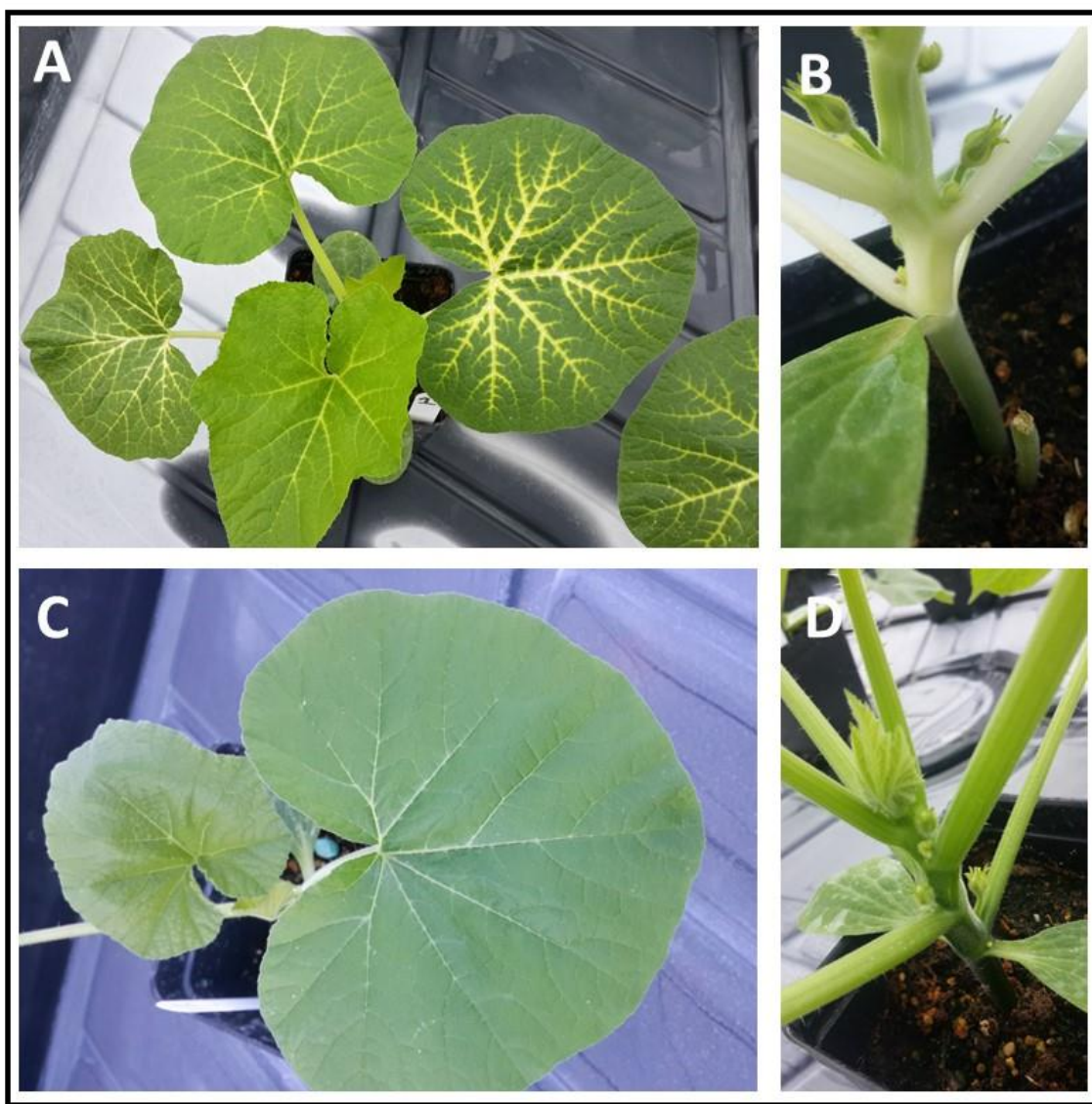


Figure 4.S7. Leaf and stem images of the squash plants in the Terrazole 35WP treatment (A and B) and the non-inoculated control treatment (–Control, C and D).

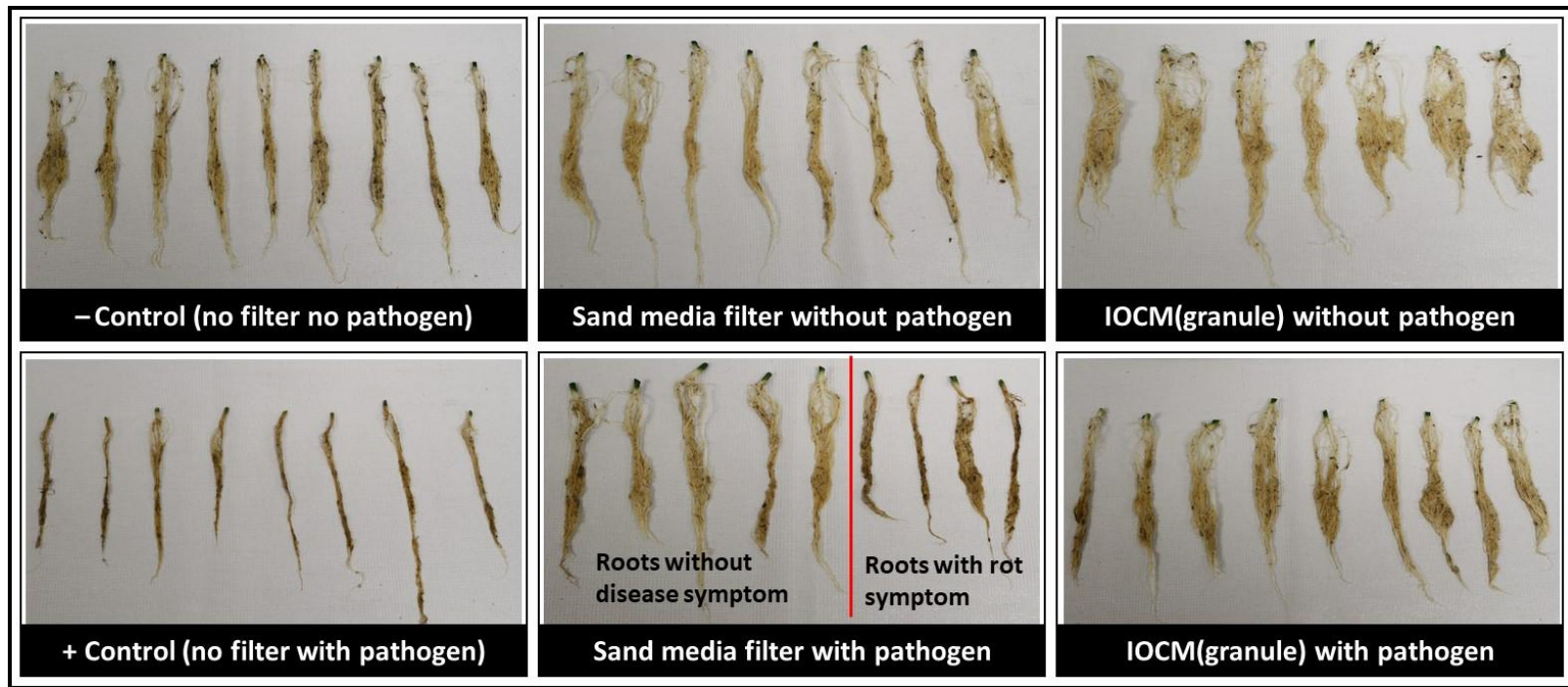


Figure 4.S8. Root images at the end of the experiment A (i.e., 11 days after inoculation).

S1. Squash Greenhouse Experiments Raw Data

Table 4.S5. Experiment A: Squash foliar fresh weight (g)

Number	- Control	Sand filter no <i>P.</i>	IOCM filter no <i>P.</i>	No filter with <i>P.</i>	Sand filter with <i>P.</i>	IOCM filter with <i>P.</i>
1	32.8	34.3	36.2	6.9	10.1	20.4
2	34.7	39.1	38.1	5.0	23.7	33.4
3	33.7	35.3	40.3	15.1	33.4	34.5
4	38.6	34.9	30.2	8.7	20.4	31.4
5	27.4	31.3	36.8	9.1	10.8	28.6
6	35.2	36.0	39.5	7.0	22.5	35.3
7	34.7	35.8	38.2	10.9	19.1	36.8
8	22.6	39.3	N.D	9.7	31.8	27.6
9	32.9	N.D	N.D	N.D	29.4	30.7
Mean	32.5	35.7	37.0	9.1	22.4	31.0
SD	4.5	2.4	3.1	2.9	7.9	4.7

Table 4.S6. Experiment A: Squash foliar dry weight (g)

Number	- Control	Sand filter no <i>P.</i>	IOCM filter no <i>P.</i>	No filter with <i>P.</i>	Sand filter with <i>P.</i>	IOCM filter with <i>P.</i>
1	2.5	2.7	2.8	1.0	1.3	1.8
2	2.5	2.9	2.9	0.9	2.1	2.6
3	2.5	2.7	3.1	1.5	2.7	2.9
4	2.8	2.6	2.3	1.1	2.1	2.5
5	2.0	2.2	2.9	1.1	1.3	2.2
6	2.6	2.7	3.1	0.8	2.1	2.7
7	2.5	2.7	2.9	1.4	1.7	2.8
8	2.6	2.9	N.D	0.9	2.5	2.2
9	2.5	N.D	N.D	N.D	2.5	2.4
Mean	2.5	2.7	2.8	1.1	2.0	2.4
SD	0.2	0.2	0.3	0.2	0.5	0.3

continue

Table 4.S7. Experiment A: Squash root fresh weight (g)

Number	- Control	Sand filter no <i>P.</i>	IOCM filter no <i>P.</i>	No filter with <i>P.</i>	Sand filter with <i>P.</i>	IOCM filter with <i>P.</i>
1	3.9	4.8	5.4	0.5	1.1	3.5
2	4.8	4.2	5.6	0.4	3.3	4.5
3	3.8	3.7	6.2	1.0	4.2	5.4
4	4.2	4.2	4.5	0.7	4.2	5.7
5	3.0	3.3	4.6	0.8	2.1	3.6
6	4.9	3.9	5.8	0.8	1.0	4.6
7	4.3	4.3	4.9	0.8	2.4	5.4
8	3.0	4.4	N.D	1.0	2.8	4.4
9	4.3	N.D	N.D	N.D	3.7	4.8
Mean	4.0	4.1	5.3	0.7	2.8	4.7
SD	0.6	0.4	0.6	0.2	1.1	0.7

Table 4.S8. Experiment A: Squash root dry weight (g)

Number	- Control	Sand filter no <i>P.</i>	IOCM filter no <i>P.</i>	No filter with <i>P.</i>	Sand filter with <i>P.</i>	IOCM filter with <i>P.</i>
1	0.33	0.32	0.39	0.11	0.20	0.26
2	0.32	0.33	0.40	0.08	0.34	0.34
3	0.29	0.27	0.44	0.18	0.32	0.37
4	0.33	0.30	0.31	0.13	0.35	0.40
5	0.24	0.26	0.37	0.13	0.32	0.28
6	0.36	0.28	0.42	0.12	0.16	0.32
7	0.36	0.31	0.39	0.15	0.32	0.41
8	0.22	0.34	N.D	0.13	0.30	0.31
9	0.34	N.D	N.D	N.D	0.34	0.35
Mean	0.31	0.30	0.39	0.13	0.29	0.34
SD	0.05	0.03	0.04	0.03	0.06	0.05

continue

Table 4.S9. Experiment B: Squash foliar fresh weight (g)

Number	- Control	+ Control	Sand filter	IOCM filter	Etridiazole
1	108.1	2.3	21.2	59.3	48.1
2	111.0	1.2	9.8	50.5	46.0
3	106.8	1.1	3.5	70.7	41.6
4	96.4	1.9	6.2	61.7	43.0
5	91.9	1.5	28.0	56.1	39.5
6	114.9	1.4	14.8	31.6	38.1
7	111.8	1.5	12.8	61.5	41.0
8	92.7	1.2	23.1	67.5	41.0
9	103.1	1.7	21.6	62.0	41.9
10	116.5	1.5	47.3	50.6	41.5
11	98.4	2.2	8.1	52.1	41.7
12	113.2	1.5	9.3	54.7	52.1
13	113.5	1.4	12.9	64.3	41.1
14	109.3	1.9	10.9	57.6	39.9
15	111.1	2.1	10.3	51.3	33.0
Mean	106.6	1.6	16.0	56.8	42.0
SD	7.8	0.4	10.7	9.0	4.2

Table 4.S10. Experiment B: Squash foliar dry weight (g)

Number	- Control	+ Control	Sand filter	IOCM filter	Etridiazole
1	9.6	1.4	4.2	6.2	4.8
2	9.7	1.0	2.9	5.1	4.4
3	9.8	0.9	2.0	7.2	4.2
4	8.4	1.2	2.3	6.1	4.2
5	8.2	1.1	4.9	5.9	4.1
6	10.9	1.2	3.6	5.1	4.2
7	9.7	1.1	3.3	6.5	4.2
8	8.0	1.1	4.1	7.0	4.1
9	9.7	1.2	4.4	6.7	4.5
10	10.4	1.2	6.4	5.2	4.1
11	8.8	1.3	3.0	5.5	4.1
12	10.6	1.0	3.6	5.9	5.1
13	10.2	0.9	4.1	7.0	4.2
14	9.5	1.3	3.6	6.1	3.9
15	10.6	1.3	3.4	5.7	3.6
Mean	9.6	1.1	3.7	6.1	4.2
SD	0.9	0.1	1.0	0.7	0.3

continue

REFERENCES

REFERENCES

1. FCC *The North American Greenhouse Vegetable Industry*; Zbeetnoff Agro-Environmental Consulting; 2006.
2. van Lenteren, J. C., A greenhouse without pesticides: fact or fantasy? *Crop Prot* **2000**, 19, (6), 375-384.
3. Pilkington, L. J.; Messelink, G.; van Lenteren, J. C.; Le Mottee, K., "Protected Biological Control"—Biological pest management in the greenhouse industry. *Biological Control* **2010**, 52, (3), 216-220.
4. Enzenbacher, T.; Hausbeck, M. In *Isolates of Phytophthora capsici differ in their ability to cause disease on cucurbit fruits*, Phytopathology, 2010; AMER PHYTOPATHOLOGICAL SOC 3340 PILOT KNOB ROAD, ST PAUL, MN 55121 USA: 2010; pp S34-S34.
5. Granke, L. L.; Quesada-Ocampo, L. M.; Hausbeck, M. K., Differences in virulence of *Phytophthora capsici* isolates from a worldwide collection on host fruits. *Eur J Plant Pathol* **2012**, 132, (2), 281-296.
6. Ristaino, J. B.; Johnston, S. A., Ecologically based approaches to management of *Phytophthora* blight on bell pepper. *Plant Dis* **1999**, 83, (12), 1080-1089.
7. Sholberg, P.; Walker, M.; O'gorman, D.; Jespersen, G., First report of *Phytophthora capsici* on cucurbits and peppers in British Columbia. *Canadian Journal of Plant Pathology* **2007**, 29, (2), 153-158.
8. Hong, C. X.; Moorman, G. W., Plant pathogens in irrigation water: Challenges and opportunities. *Crit. Rev. Plant Sci.* **2005**, 24, (3), 189-208.
9. Stephens, C.; Herr, L.; Schmitthenner, A.; Powell, C., Sources of *Rhizoctonia solani* and *Pythium* spp. in a bedding plant greenhouse. *Plant Dis* **1983**, 67, (3), 272-275.
10. Zheng, J.; Sutton, J.; Yu, H., Interactions among *Pythium aphanidermatum*, roots, root mucilage, and microbial agents in hydroponic cucumbers. *Canadian Journal of Plant Pathology* **2000**, 22, (4), 368-379.
11. Olson, H. A.; Benson, D. M., Characterization of *Phytophthora* spp. on Floriculture Crops in North Carolina. *Plant Dis* **2011**, 95, (8), 1013-1020.
12. Meador, D. P.; Fisher, P. R.; Harmon, P. F.; Peres, N. A.; Teplitski, M.; Guy, C. L., Survey of physical, chemical, and microbial water quality in greenhouse and nursery irrigation water. *HortTechnology* **2012**, 22, (6), 778-786.

13. Hong, C. X.; Richardson, P. A.; Kong, P.; Bush, E. A., Efficacy of chlorine on multiple species of *Phytophthora* in recycled nursery irrigation water. *Plant Dis* **2003**, *87*, (10), 1183-1189.
14. Stanghellini, M. E.; Rasmussen, S. L., Identification and Origin of Plant-Pathogenic Microorganisms in Recirculating Nutrient Solutions. *Adv Space Res* **1994**, *14*, (11), 349-355.
15. Hausbeck, M. K.; Lamour, K. H., *Phytophthora capsici* on vegetable crops: research progress and management challenges. *Plant Dis* **2004**, *88*, (12), 1292-1303.
16. Gevens, A.; Donahoo, R.; Lamour, K.; Hausbeck, M., Characterization of *Phytophthora capsici* from Michigan surface irrigation water. *Phytopathology* **2007**, *97*, (4), 421-428.
17. Erwin, D. C.; Ribeiro, O. K., *Phytophthora diseases worldwide*. American Phytopathological Society (APS Press): 1996.
18. Stanghellini, M.; Nielsen, C.; Kim, D.; Rasmussen, S.; Rorbaugh, P., Influence of sub-versus top-irrigation and surfactants in a recirculating system on disease incidence caused by *Phytophthora* spp. in potted pepper plants. *Plant Dis* **2000**, *84*, (10), 1147-1150.
19. van Os, E. A., Comparison of Some Chemical and Non-Chemical Treatments to Disinfect a Recirculating Nutrient Solution. *International Symposium on Soilless Culture and Hydroponics* **2009**, *843*, 229-234.
20. Lamour, K.; Hausbeck, M., Mefenoxam insensitivity and the sexual stage of *Phytophthora capsici* in Michigan cucurbit fields. *Phytopathology* **2000**, *90*, (4), 396-400.
21. Raudales, R. E.; Parke, J. L.; Guy, C. L.; Fisher, P. R., Control of waterborne microbes in irrigation: A review. *Agr Water Manage* **2014**, *143*, 9-28.
22. Calvo-Bado, L. A.; Pettitt, T. R.; Parsons, N.; Petch, G. M.; Morgan, J. A. W.; Whipps, J. M., Spatial and temporal analysis of the microbial community in slow sand filters used for treating horticultural irrigation water. *Applied and environmental microbiology* **2003**, *69*, (4), 2116-2125.
23. Martínez, F.; Castillo, S.; Carmona, E.; Avilés, M., Dissemination of *Phytophthora cactorum*, cause of crown rot in strawberry, in open and closed soilless growing systems and the potential for control using slow sand filtration. *Scientia horticultrae* **2010**, *125*, (4), 756-760.
24. Meador et al., Survey of physical, chemical, and microbial water quality in greenhouse and nursery irrigation water. *HortTechnology* **2012**, *22*(6), 778-786.
25. Ehret, D. L.; Alsanius, B.; Wohanka, W.; Menzies, J. G.; Utkhede, R.,

- Disinfestation of recirculating nutrient solutions in greenhouse horticulture. *Agronomie* **2001**, 21, (4), 323-339.
26. Lee, E.; Oki, L. R., Slow sand filters effectively reduce Phytophthora after a pathogen switch from Fusarium and a simulated pump failure. *Water Res* **2013**, 47, (14), 5121-5129.
 27. Ufer, T., Werres, S. K., Posner, M., and Wessels, H.-P., Filtration to eliminate Phytophthora spp. from recirculating water systems in commercial nurseries. *Online Plant Health Progress* **2008**, <http://dx.doi.org/10.1094/PHP-2008-0314-01-RS>.
 28. van Os, E. A.; Amsing, J. J.; van Kuik, A. J.; Willers, H., Slow sand filtration: A potential method for the elimination of pathogens and nematodes in recirculating nutrient solutions from glasshouse-grown crops. *International Symposium on Growing Media and Hydroponics, Vols I and II* **1998**, (481), 519-526.
 29. Déniel, F.; Rey, P.; Chérif, M.; Guillou, A.; Tirilly, Y., Indigenous bacteria with antagonistic and plant-growth-promoting activities improve slow-filtration efficiency in soilless cultivation. *Canadian journal of microbiology* **2004**, 50, (7), 499-508.
 30. Jeon, S.; Krasnow, C. S.; Kirby, C. K.; Granke, L. L.; Hausbeck, M. K.; Zhang, W., Transport and Retention of Phytophthora capsici Zoospores in Saturated Porous Media. *Environmental Science & Technology* **2016**, 50, (17), 9270-9278.
 31. Van Os, E.; Bruins, M.; Wohanka, W.; Seidel, R. In *Slow filtration: a technique to minimise the risks of spreading root-infecting pathogens in closed hydroponic systems*, V International Symposium on Protected Cultivation in Mild Winter Climates: Current Trends for Sustainable Technologies 559, 2000; 2000; pp 495-502.
 32. Koné, D.; Csinos, A.; Jackson, K.; Ji, P., Evaluation of systemic acquired resistance inducers for control of Phytophthora capsici on squash. *Crop Prot* **2009**, 28, (6), 533-538.
 33. Jeon, S.; Krasnow, C. S.; Bhalsod, G. D.; Harlan, B.; Hausbeck, M. K.; Safferman, S. I.; Zhang, W., Filtration of Pythium aphanidermatum zoospores in recycled irrigation water to control poinsettia disease in greenhouses. *J. Agric. Food Chem.* **2017**, (in review).
 34. MetaMateria Phosphorus capture & recovery. <http://www.metamateria.com/phosphorus-capture-and-recovery> (Last accessed on July 13, 2017),
 35. Gadgil, A., Drinking water in developing countries. *Annual review of energy and the environment* **1998**, 23, (1), 253-286.

36. Huisman, L.; Wood, W., *Slow sand filtration*. World Health Organization Geneva: 1974; Vol. 16.
37. WHO Rapid Sand Filtration. (Fact Sheets on Environmental Sanitation). http://www.who.int/water_sanitation_health/hygiene/emergencies/fs2_14.pdf (May 27),
38. Stewart-Wade, S. M., Plant pathogens in recycled irrigation water in commercial plant nurseries and greenhouses: their detection and management. *Irrigation Sci* **2011**, 29, (4), 267-297.
39. Zhang, W.; Isaacson, C. W.; Rattanaudompol, U.; Powell, T. B.; Bouchard, D., Fullerene nanoparticles exhibit greater retention in freshwater sediment than in model porous media. *Water Res.* **2012**, 46, (9), 2992-3004.
40. Abudalo, R. A.; Bogatsu, Y. G.; Ryan, J. N.; Harvey, R. W.; Metge, D. W.; Elimelech, M., Effect of ferric oxyhydroxide grain coatings on the transport of bacteriophage PRD1 and *Cryptosporidium parvum* oocysts in saturated porous media. *Environ. Sci. Technol.* **2005**, 39, (17), 6412-6419.
41. Wallace, A.; Romney, E.; Alexander, G.; Kinnear, J., Phytotoxicity and some interactions of the essential trace metals iron, manganese, molybdenum, zinc, copper, and boron. *Communications in Soil Science & Plant Analysis* **1977**, 8, (9), 741-750.
42. Bartakova, I.; Kummerova, M.; Mandl, M.; Pospisil, M., Phytotoxicity of iron in relation to its solubility conditions and the effect of ionic strength. *Plant and soil* **2001**, 235, (1), 45-51.
43. Mohanram, A.; Ray, C.; Harvey, R. W.; Metge, D. W.; Ryan, J. N.; Chorover, J.; Eberl, D. D., Comparison of transport and attachment behaviors of *Cryptosporidium parvum* oocysts and oocyst-sized microspheres being advected through three minerologically different granular porous media. *Water Res.* **2010**, 44, (18), 5334-5344.
44. Kim, S.-B.; Park, S.-J.; Lee, C.-G.; Kim, H.-C., Transport and retention of *Escherichia coli* in a mixture of quartz, Al-coated and Fe-coated sands. *Hydrological Processes* **2008**, 22, (18), 3856-3863.
45. Uchida, R., Essential nutrients for plant growth: nutrient functions and deficiency symptoms. *Plant nutrient management in Hawaii's soils* **2000**, 31-55.
46. Darling, D., A mobile filtration plant to eliminate fungal spores in irrigation water for plant propagation. Short communication. *Australian Forest Research* **1977**.
47. Vankuik, A., Eliminating *Phytophthora cinnamomi* in a recirculated irrigation system by slow sand filtration. *Mededelingen-Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen Universiteit Gent (Belgium)* **1994**.

48. Krasnow, C. S.; Hammerschmidt, R.; Hausbeck, M. K., Characteristics of Resistance to Phytophthora Root and Crown Rot in Cucurbita pepo. *Plant Dis* **2017**, *101*, (5), 659-665.
49. Babadoost, M., Phytophthora blight: a serious threat to cucurbit industries. *Urbana* **2004**, *51*, 61801.
50. Ellis, K.; Wood, W., Slow sand filtration. *Critical Reviews in Environmental Science and Technology* **1985**, *15*, (4), 315-354.
51. Dumroese, R.; James, R.; Wenny, D., Trial of a granular etridiazole and thiophanate-methyl mixture to control Fusarium root disease of container-grown Douglas-fir seedlings. *New Forests* **1990**, *4*, (3), 231-236.
52. Kuthubutheen, A.; Wickneswari, R.; Kumar Das, V., Efficacy of selected triorganotin (IV) compounds on leaves against Phytophthora palmivora (Butler) Butler isolated from black pepper and cocoa. *Applied organometallic chemistry* **1989**, *3*, (3), 243-248.
53. Snyman, C.; Kotzé, J., Evaluation of application techniques of four fungicides for the control of Phytophthora root rot on avocado seedlings. *S Afr Avocado Growers' Assoc Yrb* **1983**, *6*, 79-81.
54. McCain, A.; Byrne, T., Chemical control of pythium root rot in ornamentals with Dexon and Terrazole. *California Agr.* **1966**, *20*, (5), 14-16.
55. Moorman, G.; Kim, S., Species of *Pythium* from greenhouses in Pennsylvania exhibit resistance to propamocarb and mefenoxam. *Plant Dis.* **2004**, *88*, (6), 630-632.
56. Raabe, R.; Hurlimann, J.; Byrne, T., Chemical and biological approaches to control of pythium root rot of poinsettia. *California Plant Pathol.* **1981**, *52*, 5-8.
57. Benson, D. M., Efficacy and in vitro activity of two systemic acylalanines and ethazole for control of *Phytophthora cinnamomi* root rot of azalea (*Rhododendron obtusum*). *Phytopathology* **1979**, *69*, 174-178.
58. Stephens, C.; Stebbins, T., Control of damping-off pathogens in soilless container media. *Plant Dis.* **1985**, *69*, (6), 494-496.
59. Lamour, K. H.; Daughtrey, M. L.; Benson, D. M.; Hwang, J.; Hausbeck, M. K., Etiology of *Phytophthora drechsleri* and *P. nicotianae* (= *P. parasitica*) diseases affecting floriculture crops. *Plant Dis.* **2003**, *87*, (7), 854-858.
60. Moorman, G.; Kang, S.; Geiser, D.; Kim, S., Identification and characterization of *Pythium* species associated with greenhouse floral crops in Pennsylvania. *Plant Dis.* **2002**, *86*, (11), 1227-1231.

61. Ferrin, D.; Rohde, R., In vivo expression of resistance to metalaxyl by a nursery isolate of *Phytophthora parasitica* from *Catharanthus roseus*. *Plant Dis.* **1992**, 76, (1), 82-84.
62. Garzón, C. D.; Molineros, J. E.; Yáñez, J. M.; Flores, F. J.; del Mar Jiménez-Gasco, M.; Moorman, G. W., Sublethal doses of mefenoxam enhance pythium damping-off of geranium. *Plant Dis.* **2011**, 95, (10), 1233-1238.
63. Meyer, M.; Hausbeck, M., Using soil-applied fungicides to manage *Phytophthora* crown and root rot on summer squash. *Plant Dis* **2013**, 97, (1), 107-112.

CHAPTER 5

CONCLUSION AND FUTURE WORK

In this work, effective methods for the vegetable and floriculture industries to treat irrigation water and remove and/or reduce water mold pathogens (*Phytophthora* and *Pythium* zoospores) were investigated, as well as the pathogen retention mechanisms in porous media. A laboratory column study was performed to investigate the effect of filter media properties, solution chemistry, and zoospore encystment on the zoospore transport. Greenhouse experiments were then conducted to demonstrate the proof-of-concept of using fast-flow filtration systems to control the pathogen infections in susceptible host plants (i.e., poinsettia and squash). Major conclusions were outlined as follows.

I. The transport of *Phytophthora capsici* zoospores in porous media was collectively controlled by surface properties of zoospores and porous media, and solution chemistry. Significantly more encysted zoospores were retained in IOCS than in uncoated sand, and at pH 4.4 than at pH 7.2, which likely resulted from increased electrostatic attraction between zoospores and grain surfaces. At pH 7.2, up to 99% and 96% of the encysted zoospores were removed in IOCS and uncoated sand, respectively, due to a combination of strong surface attachment, pore straining, and adhesive interactions. Motile biflagellate zoospores were more readily transported than encysted zoospores, thus posing a greater dispersal and infection risk.

II. The fast-flow sand filtration with low water pressure consistently removed *Pythium aphanidermatum* zoospores, and no differences in root rot severity, height, biomass, and horticultural rating were observed for the plants in the sand filter treatment, compared with the non-inoculated control plants. However, the activated carbon filter

removed essential nutrients from the irrigation water, resulting in plant nutrient deficiency and consequently leaf chlorosis and lower plant biomass, height, and horticultural ratings. Overall, the rapid sand filter has the potential to be used for controlling *Pythium* root rot in greenhouses, but maintenance was required to prevent clogging.

III. The IOCM filter could effectively protect squash plants from *Phytophthora capsici*, but caused the nutrient deficiency in the squash. The sand filter could not prevent, but only slow the disease development in the squash. The IOCM filter may be used to control *Phytophthora* infections in greenhouse vegetable production if the nutrients can be supplied separately instead of through irrigation water.

Overall, the results suggest that physical removal of pathogens using fast-flow filtration can overcome many limitations of fungicide application, and may be a promising alternative for disease management in greenhouses. Building upon this work, future research will need to focus on assessing the longevity of the system performance by optimizing filter media and operation parameters. The filter design can be further improved to reduce clogging of the sand filter. Operation parameters such as irrigation frequency and duration can be optimized to provide adequate water to crops while minimizing the pathogen infection and improving the longevity of the filtration systems. Finally, future work should be conducted in large-scale systems to assess the performance, optimize operation parameters, and analyze operation costs in commercial greenhouses.