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Slow sand filters effectively reduce *Phytophthora* after a pathogen switch from *Fusarium* and a simulated pump failure

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ABSTRACT

Slow sand filtration has been shown to effectively reduce *Phytophthora* zoospores in irrigation water. This experiment tested the reduction of *Phytophthora* colony forming units (CFUs) by slow sand filtration systems after switching the pathogen contaminating plant leachate from *Fusarium* to *Phytophthora* and the resilience of the system to a short period without water, as might be caused by a pump failure. The slow sand filtration system greatly reduced *Phytophthora* CFUs and transmission after switching the pathogens. In addition, *Phytophthora* reduction by the slow sand filter was equally effective before and after the simulated pump failure. Reduction of *Fusarium* was not seen by the SSFs, before or after the simulated pump failure. The results suggest that slow sand filters are effective at reducing larger organisms, such as *Phytophthora* zoospores, even after a pump failure or a change in pathogens.

1. Introduction

Slow filters are low maintenance systems that do not require special equipment. Generally, a slow filter consists of two tanks, a pump, and the filter medium. The water to be disinfested is pumped from the first tank through the filter medium at a low flow rate (10–20 cm hr^{-1}) into the second tank (Ehret et al., 2001). The filtered water is cleaned by its passage through the filter medium, due to a poorly understood combination of physical, chemical, and biological mechanisms (Weber-Shirk and Dick, 1997a,b). Slow filtration was first developed as a purification system for potable drinking water; one of the earliest recorded uses of slow sand filtration helped prevent a cholera outbreak in a city using the a slow sand filter system to purify drinking water (Huisman and Wood, 1974). As newer methods of water purification developed, slow filtration was set aside or used in tandem with other systems (Huisman and Wood, 1974; Logsdon et al., 2002). Now, slow filtration is seeing expanded use to remove plant pathogens from recirculating irrigation systems. A variety of media can be used for slow filtration, including sand, pozzalana grains, and rockwool; this study focuses on slow sand filters (SSF).

An established SSF has been shown to reduce a wide range of plant pathogens including *Phytophthora* spp. (Garibaldi et al., 2003; Grasso et al., 2003), *Pythium* spp. (Deniel et al., 2004), *Xanthomonas campestris* (Brand and Wohanka, 2001), and the

Abbreviations: CFU, colony forming unit; SSF, slow sand filter.

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Radopholus similis nematode (van Os et al., 1999) in closed circulating systems. In addition, other reports have shown a well-established SSF can remove a variety of *Phytophthora* spp. at once; slow sand filtration was able to remove the *Phytophthora* spp. from creek water that had tested positive for several years for various *Phytophthora* spp., including *Phytophthora ramorum* (Harris, 2012).

The greater role of biologically-mediated pathogen reduction is what makes SSFs differ from rapid sand filters. While both types of sand filtration rely on physical-chemical reduction mechanisms, as evidenced by how even a brand new SSF shows some reduction efficacy, pathogen reduction by slow sand filtration also relies heavily on biological mechanisms (Weber-Shirk and Dick, 1997b). The reduction efficacy increases when the SSF has had time to establish its biological community, whereas rapid sand filters do not generally show increased filtration efficacy over time (Alsanius et al., 2001). The density of that biological community in an SSF is correlated with the reduction efficacy (Campos et al., 2002) and elimination of the biological community has a significantly negative impact on reduction efficacy (Weber-Shirk and Dick, 1997a). The biological components are not well-understood; SSF systems develop a layer of microbes, called the "filter skin" (German, *schmutzdecke*), which is key to the high efficacy of pathogen reduction (Ellis, 1985). Pathogens are trapped and broken down by these microbes, probably as food; the trapping is from a combination of physical filtration and adsorption, influenced by a variety of factors reviewed earlier (Stevik et al., 2004). Scanning electron micrographs and molecular analyses show an SSF's biological community represents a diverse microbial community that forms the biofilm (Calvo-Bado et al., 2003; Joubert and Pillay, 2008). Biofilms can provide protection to their members, increasing their resilience against stresses such as drought (Rittmann, 2007; Singh et al., 2006).

A critical point and a potential vulnerability in an SSF system is the water supply pump which is vulnerable to various mechanical problems. Slow filters are designed to have a constant stream of liquid passing through them, providing water, nutrients, and oxygen to the microbes; if the pump fails, then this supply stops. Even a short pump failure could negatively impact the effectiveness in filtering, but no studies have been done to measure the impact. If an SSF in use at a nursery or greenhouse is compromised by a pump failure, then the filtered water would be unsafe for use. However, the biofilm may help protect the microbes, so a pump failure may not affect reduction efficacy.

The wide range of functional groups present in the SSF's *schmutzdecke* community allows utilization of a wide range of organisms and materials as sources of food (Aslan and Cakici, 2007; Mine et al., 2003; Renault et al., 2012). Given the importance of biological removal in the SSF's reduction mechanism, it seems likely that a slow sand filter may need to be adapted to the specific challenges presented by distinct pathogens; however, this has not been demonstrated extensively in a controlled setting.

Part of this study tested the adaptability of the SSF, by challenging the SSF with two distinctly different plant pathogens, *Phytophthora capsici* and *Fusarium oxysporum* f. sp. *Lycopersici*. These two organisms were chosen because of their phylogenetic differences (an oomycete and a fungus), chemical differences (cell walls of β1, 3 glucans in the oomycetes and cell walls of chitin in the fungi), and because both pathogens are readily spread by water (Hong and Moorman, 2005). In addition, previous studies indicate large differences in the effectiveness of SSF in removing these two pathogens. Slow sand filtration has a history of effectively removing a variety of *Phytophthora* spp. without special preparations (Stewart-Wade, 2011). For SSFs to effectively remove *F. oxysporum*, special preparation is needed, such as the addition of fungal cell wall preparations or *Fusarium* spp. antagonists; the specific mechanisms of why special preparations are needed are not clear, though it may be related to the microbial community. Even with these preparations, reduction efficacy of *F. oxysporum* is generally not as a high as for *Phytophthora* spp. (Brand and Wohanka, 2001; Ehret et al., 1999).

The purpose of this study was to examine the adaptability of established SSFs by challenging them with different biotic and abiotic stressors. The biotic challenge was to remove two different plant pathogens fed in series, *P. capsici* and *F. oxysporum f. sp. Lycopersici*. The abiotic challenge was a pump failure, monitoring the pathogen colony forming unit (CFU) reduction by the SSF system after a simulated pump failure. It was predicted the pathogen reduction efficacy would be reduced during either scenario, with the SSF making a full recovery to its maximum potential reduction efficacy given enough time.

2. Materials and methods

2.1. Slow sand filter design

Thirteen slow sand filters were utilized for the experiment. Each filter was made of two 1 m sections of 10.16 cm (4 in) diameter PVC pipe, joined end-to-end with a flange. A 0.635 cm (¼ in) diameter hole was drilled into one length of pipe and a sampling port was inserted; the pre-SSF sample was taken from this sampling port. A second sampling port was set beneath the column, under the filter media; the SSF-filtered samples were from this sampling port (Fig. 1). The bottom flange was layered with a size gradient of rocks and pebbles. In order from bottommost to upmost, the layers were: small rocks (ca. 3 cm), large aquarium pebbles (Kordon LLC, Hayward, CA), smaller pebbles (Kordon LLC, Hayward, CA), #2-/16 coarse sand (RMC Pacific Materials, Inc., Pleasanton, CA), #3 coarse sand (RMC Pacific Materials, Inc., Pleasanton, CA), and #60 sand lapis luster, quartz sand (RMC Pacific Materials, Inc., Pleasanton, CA); the uppermost, fine sand was the same as the filtering media. Each layer was just thick enough to cover the layer below.

The bottom half of each SSF was filled with about 1 m (39.37 in) depth of #60 quartz lustre sand (RMC Pacific Materials, Inc., Pleasanton, CA). The sand was thoroughly rinsed with water and the supernatant was decanted several times, to remove fine particulate matter. The washed sand was dried in a drying oven with continuous heated airflow. The top half of the filter was filled with leachate collected from common nursery plants to a depth of about 1 m above the sand surface, providing a pressure head to push the leachate through the