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Elimination of *Tobacco mosaic virus* from irrigation runoff using slow sand filtration

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ABSTRACT

Slow sand filters (SSF) are effective in removing pythiacious organisms from captured runoff to prepare the water for reuse in irrigation. Plant viruses can also be present in this runoff, but until this study, it hadn't been clearly shown that SSFs can remove these pathogens. In this study, purified preparations of *Tobacco mosaic virus* (TMV) were regularly added to captured irrigation runoff water that was provided to the SSFs. Water samples were collected weekly from sampling ports above and below the sand bed and tested for the presence of TMV by ELISA and inoculation to indicator plants. Biologically viable TMV passed through the sand filters for approximately 5 weeks, followed by a gradual reduction and then elimination of detectable amounts of the virus by week 6–9. Testing of water samples continued for several more weeks after the initial loss of virus titer to confirm complete elimination. This is the first controlled study showing elimination of a plant virus from captured irrigation runoff using SSFs. Since TMV is a highly robust organism, it is likely that other plant pathogenic viruses can also be removed using SSF systems.

1. Introduction

Irrigation water, from both fresh and recycled sources, is known to vector all types of plant pathogens (fungi and oomycetes, bacteria, viruses, and nematodes) to nursery and greenhouse crops (Hong and Moorman, 2005). Water treatment is necessary to prevent the spread of disease into and within plant production facilities and can be accomplished by many different methods, including chemical (such as chlorine and ozone), pasteurization with heat, UV radiation, membrane filtration, or bio-filtration (Ehret et al., 2001). Construction, operation, and energy costs can be very high to treat the large volumes of water used for irrigation by greenhouse and nursery operations (Stewart-Wade, 2011). However, slow sand filters are a low-cost and low-energy water filtration system utilizing biological processes to treat the water (Ufer et al., 2008).

Slow sand filters (SSF) have been used for over 200 years as a system for purifying water (Haig et al., 2011) and helped prevent a cholera outbreak in 1892 in Altona, Germany (Ehret et al., 2001). SSFs are widely used in greenhouse and nursery plant production facilities in Europe (Barth, 1997). They rely on the establishment of a diverse microorganism community on the sand surface, consisting of algae, bacteria, fungi, actinomycetes, protozoa, rotifers, nematodes, and flatworms (Calvo-Bado et al., 2003; Stewart-Wade, 2011) for pathogen reduction efficacy. This microorganism community is referred to as the "filter skin" (German, *schmutzdecke*), where pathogens are trapped and broken down by microbes (Hijnen et al., 2004). SSFs require low flow rates (10–20 cm h^{-1}) and become more effective as the *schmutzdecke* matures and/or adapts to the specific pathogen load (Lee and Oki, 2013).

SSF systems have been shown to remove a variety of plant pathogens, including *Phytophthora* spp. (Garibaldi et al., 2003; Grasso et al., 2003; Lee and Oki, 2013), *Pythium* spp. (Déniel et al., 2004), *Fusarium* spp. (Déniel et al., 2004), *Xanthomonas campestris* (Brand and Wohanka, 2001), and *Radopholus similis* nematode (van Os et al., 1998). Some work has shown human pathogenic virus and bacteriophage elimination from drinking water via SSFs (Elliott

Abbreviations: SSF, slow sand filter; TMV, *Tobacco mosaic virus*; ELISA, enzyme-linked immunosorbent assay; PFBV, *Pelargonium flower break virus*; ToMV, *Tomato mosaic virus*.

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et al., 2011; Elliott et al., 2008; McConnell et al., 1984), but complete removal of plant pathogenic viruses has not been documented (van Os et al., 1998). Krczal et al. (1995) found a decrease in *Pelargonium flower break virus* (PFBV) concentration within the solution and a delayed infection of *Pelargonium* plants as a result of SSF in a recirculating nutrient solution. As viruses are found in both fresh and recycled water sources used for irrigation at plant production facilities (Berkelmann et al., 1995; Koenig, 1986; Paludan, 1985; Pares et al., 1992), determining the effectiveness of SSFs to remove viruses would be helpful information for growers deciding whether a SSF system is the optimum treatment technology for their operation.

The work presented here investigates the effectiveness of SSFs to remove *Tobacco mosaic virus* (TMV) from recycled irrigation water in a greenhouse. TMV is a member of the genus *Tobamovirus* of plant viruses with rod shaped particles and ssRNA(+) genomes. TMV rods are about 300 nm × 18 nm in size with 6400 nucleotides of RNA on average, depending on the strain. TMV is one of the most serious threats to plants in greenhouses due to extreme stability, ease of mechanical transmission, and severe symptom expression in a large number of hosts (Scholthof, 2004). TMV has been found in irrigation water, usually from virus release by infected plant roots (Park et al., 1999; Yarwood, 1960), and has longevity of sap infectivity in vitro for up to 3000 days (Mehle and Ravnikar, 2012). For these reasons, TMV is commonly used as a model system for many plant virus research studies. If a removal method is successful with TMV, it is likely to be successful with viruses that are less stable and more difficult to transmit. We report here the successful elimination of TMV from water using slow sand filtration.

2. Materials and methods

2.1. Filter preparation

SSF columns were composed of two ~1 m sections of 10.16 cm (4 in) diameter PVC pipe, joined end-to-end with a central flange. Water sampling ports (Model C-13074-30 Cole-Parmer, Vernon Hills, IL) were located immediately above (pre-filtered water) and below (filtered water) the sand bed as shown in Fig. 1. The bottom flange fitting was layered with a size gradient of rocks and pebbles to support the filter sand in the columns, with the largest particles on the bottom. The aggregate, in order from bottommost to topmost, were small stones (ca. 3 cm), large aquarium pebbles (Kordon LLC, Hayward, CA), small aquarium 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 finally, #60 sand lapis luster quartz sand (RMC Pacific Materials, Inc., Pleasanton, CA) which is the same as the filtering media (Lee and Oki, 2013). The sand filtration media was prepared by thoroughly rinsing with water and decanting the supernatant several times to remove fine particulate matter. The washed sand was air dried, then used to fill the bottom section of the column between the two sampling ports as shown in Fig. 1.

The SSF columns were located in greenhouse facilities at the University of California Agriculture and Natural Resources South Coast Research and Extension Center in Irvine, CA. Eighty pepper plants (*Capsicum annum* 'Anaheim') were grown in the same unheated greenhouse in a peat-based medium (Sunshine #3, SunGro Horticulture, Agawam, MA) in #1 containers on a tray-type bench that allows collection of irrigation leachate. Twice-daily irrigations produced approximately 100 L of accumulated leachate runoff each day. Runoff was captured in drums under the benches to which float switches were installed to monitor the levels and interrupt irrigation if sufficient leachate was present. Leachate was continuously pumped to a manifold that distributed the water to the tops of the SSF columns, beginning 30 days prior to addition of the TMV

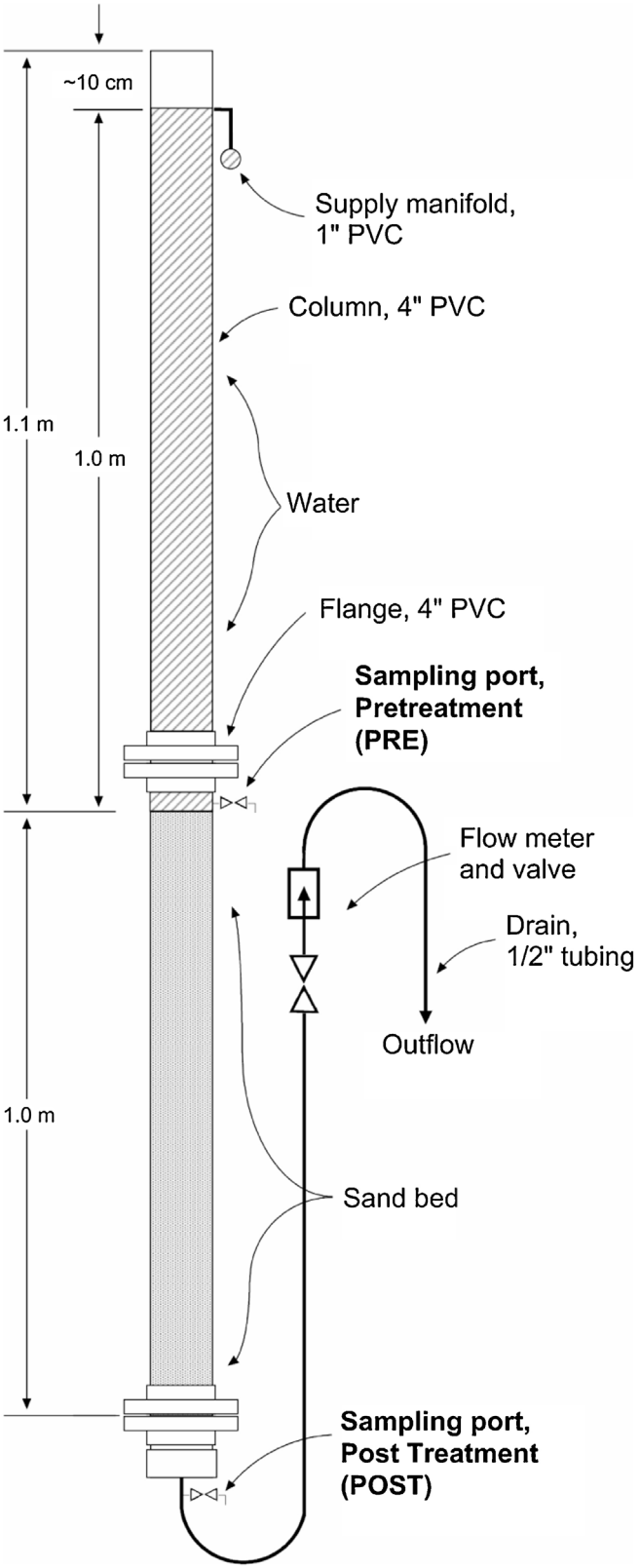

Fig. 1. Slow sand filter. The slow sand filter was constructed of 4" dia. PVC pipe parts. Water samples were collected from just above the sand bed, Pretreatment (PRE), and after water had passed through the sand bed, Post Treatment (POST).