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Authors

Younis, Bassam A
Mahoney, Laura
Schweigkofler, Wolfgang
et al.

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Inactivation of plant pathogens in irrigation water runoff using a novel UV disinfection system

Bassam A. Younis · Laura Mahoney · Wolfgang Schweigkofler · Karen Suslow

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Abstract Untreated recycled irrigation water has been shown to introduce and spread plant pathogens such as *Pythium* and *Phytophthora* in commercial nurseries. Nevertheless, few nurseries currently treat their recycled irrigation water. Instead, nurseries use prophylactic pesticides to control the spread of plant pathogens, which increases costs and promotes the growth of resistant pathogens. Of interest to California is the spread of *Phytopthora ramorum,* causal agent of Sudden Oak Death (SOD), responsible for the death of tens of thousands of trees in California and Oregon. This study investigated the use of a novel UV disinfection system to inactivate *P. ramorum* and other microbial contaminants at the National Ornamental Research Site at the Dominican University of California (NORS-DUC). In this system, the UV lamps do not come in contact with the water and hence remain free of the 'lamp fouling' problem. Tests on waters having the same characteristics as run-off from commercial nurseries showed a minimum of 3.7 log removal of bacterial species, 91.7% reduction of fungal counts, and 100% inactivation of the *P. ramorum* in the effluent. Treating the run-off from plant nurseries limits the spread of plant pathogens and enables the onsite re-use of the run-off.

B. A. Younis (✉) · L. Mahoney
Department of Civil and Environmental Engineering, University of California Davis, Davis, CA 95616, USA
e-mail: bayounis@ucdavis.edu

W. Schweigkofler · K. Suslow
Department of Science and Mathematics, Dominican University of California, San Rafael, CA 94901, USA

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Introduction

Untreated irrigation water runoff and untreated recycled irrigation water have been shown to introduce and spread microbial pathogens, such as *Pythium* and *Phytophthora*, in commercial nurseries (Ali-Shtayeh and MacDonald 1991; Hong et al. 2003; Kong et al. 2003; Hong and Moorman 2005; Werres et al. 2007). Dispersal of these pathogens is also of concern in closed hydroponic systems. These pathogens belong to the Oomycota and are often referred to as water molds that are most active during wet and humid periods and produce flagellated spores, called zoospores that can spread through the water. *Phytophthora sp*. cause diseases in agriculture, arboriculture and natural ecosystems, and the estimated losses associated with these pathogens are in the billions of dollars (Erwin and Ribeiro 1996; Lamour 2013; Jung et al. 2016).

Of particular concern in California is the spread of *P. ramorum,* causal agent of Sudden Oak Death (SOD). Costs associated with SOD incorporate costs to property owners, ornamental nursery industry, and the state and federal government. There are hundreds of thousands of susceptible oak trees located on near developed communities. Infected trees in these areas will need to be removed, disposed of, and replaced at the cost of the landowner or local government. Kovacs et al. (2011) estimated that this would amount to a discounted cost of

$7.5 million and an associated $135 million in losses to property values for single family homes in California.

Sudden Oak Death was first noticed in the 1990s when California hikers along the central coast reported oaks suddenly dying (Grünwald et al. 2012). This die-off was frequently found along the interfaces between urban and natural areas. In 2000 the pathogen causing SOD, *P. ramorum*, was isolated and researchers soon noticed that it belonged to the same species as a newly described pathogen from diseased rhododendrons and viburnums in European nurseries in 1993 (Orlikowski et al. 2007). *P. ramorum* causes foliar and shoot blight on many plants, including important ornamental species, and bleeding cankers on the tree trunks leading to the death of the plant on relatively few hosts, like coast live oak (*Quercus agrifolia*) and tan oak (*Notholithocarpus densiflorus*). This disease is responsible for the death of tens of thousands of trees in California and Oregon and most strongly affects tanoak, coast live oak, California black oak, and Shreve's oak (Rizzo and Garbelotto 2003). *P. ramorum* also causes severe damage on plantations of the non-native Japanese larch (*Larix kaempferii*) in the United Kingdom and Ireland; the disease on this new host was named 'Sudden larch death' (Grünwald et al. 2012).

Abundant inoculum can be produced on foliar hosts (e.g. *Rhododendron sp., Camellia sp*.) and spread from there to both foliar and non-foliar hosts. Due to this mode of transportation, there are instances of the *Phytopthora* species moving from ornamental nurseries to the natural environment due to uncontrolled contamination (Ghimire et al. 2011). Nevertheless, few nurseries currently treat their irrigation water (Banihashemi et al. 2010). Instead, nurseries use fungicides to control the spread of fungal pathogens and oomycetes, which increases costs and promotes the growth of resistant plant pathogens. In addition, these plant pathogens may be suppressed when under the presence of fungicides and proliferate when the fungicide is discontinued (Hong et al. 2003). For these reasons, various techniques, including the use of chlorine, ozone and UV light, have been used to mitigate the spread of the pathogen, but each technique has its drawbacks.

Most methods for the treatment of irrigation water can be costly for small operations. The most common treatment method used is liquid chlorine injection. This technique requires consistent addition, monitoring of chlorine concentrations, assessment of the system water quality and on-site storage (Hong et al. 2003; Abu-Orf et al. 2014). The chlorine dose needed is dependent on water quality because the high nitrogen and organic content in the dissolved and suspended matter incorporated in irrigation run-off increases the chlorine demand on the system. Ozone has similar limitations to using a liquid chlorine injection, but it can be generated on demand. Nevertheless, generally ozone has higher capital costs compared to the use of liquid chlorine and ultra-violet (UV) light treatment (Abu-Orf et al. 2014).

Ultraviolet light works as a disinfectant by exciting the nucleic acids in DNA and RNA. This excitation results in the dimerization of adjacent nucleic acids and prevents the further transcription of the DNA or RNA and inhibits replication. Since UV disinfection is a physical treatment process, it avoids generating toxic by-products caused by the use of oxidizing chemical disinfectants, such as chlorine. Similarly, there is no additional smell or taste added to the water, no danger of overdosing the disinfectant, and no need to store hazardous materials on site. Another benefit of UV disinfection over chlorine and ozone is that it alone is effective against both bacterial and viral pathogens.

The ability of a UV system to disinfect agricultural water run-off is dependent on its ability to deliver a UV dose sufficient to inactivate the pathogens of concern. The UV dose depends on the intensity of the UV light emitted by the lamps, on the flow rate of the water through the system, and on the UV transmittance (UVT) of the water. Factors that determine the UVT include the level of suspended solids, the turbidity, color, and the concentration of soluble organic matter. The run-off from agricultural activities is often high in dissolved and suspended matter and the resulting turbidity can thus be sufficiently high as to lower the water's transmittance of UV radiation thereby increasing, sometimes by more than 4-fold, the UV dose that is required to inactivate *Pythium* and *Phytophthora* (Banihashemi et al. 2010). Thus it is important that the water quality parameters of the untreated influent be quantified to ensure that the UVT does not fall below a level that would reduce UV light penetration and thus inhibit its germicidal ability. It is also important that the UV system itself, through aspects of its design, remains capable of delivering sufficient UV dose even when the UVT is low. One way to increase the UV dose is to reduce the flow rate of the water through the system thereby increasing the time in which the pathogens are exposed to the UV light. In many commercial

applications, this is not an acceptable remedy due to the large quantities of runoff water generated in daily operations. Moreover, by decreasing the flow rate, the resulting flow can become laminar leading to significant reduction in mixing. When mixing is reduced, the disinfection efficiency in conditions of low UVT is also reduced since the pathogens that are not brought sufficiently close to the source of the UV light can leave the system without having received sufficient dose for inactivation.

Recently, a novel system for water disinfection with UV light was developed and tested with success in the treatment of effluent from a number of municipal waste water treatment plants (Younis 2014). This system, by virtue of a number of features unique to its design, has the potential of being particularly suitable for use in agricultural and horticultural operations where the water run-off is often low in UV transmittance. The new system also benefits from being free of the problem of 'lamp fouling' that is present in most commercially-available UV systems. In such systems, the UV-emitting lamps are immersed within the water being treated and hence, with time, become covered with bio-film and mineral deposits that reduce the intensity of the emitted light and necessitate frequent system shut downs for cleaning. The objective of this study was to introduce this system to the plant-pathology community by testing it, in situ, at a facility for the study of diseases of ornamental plants to assess its performance in inactivating *P. ramorum* in actual irrigation run-off water representative of that obtained in commercial nurseries.

Materials and methods

The UV system presented here is shown in Fig. 1. Untreated water enters the system into a pressure vessel located at the bottom of the column and exits this vessel through a series of nozzles arranged along the circumference of a circle. Affixed to the top of this vessel is a

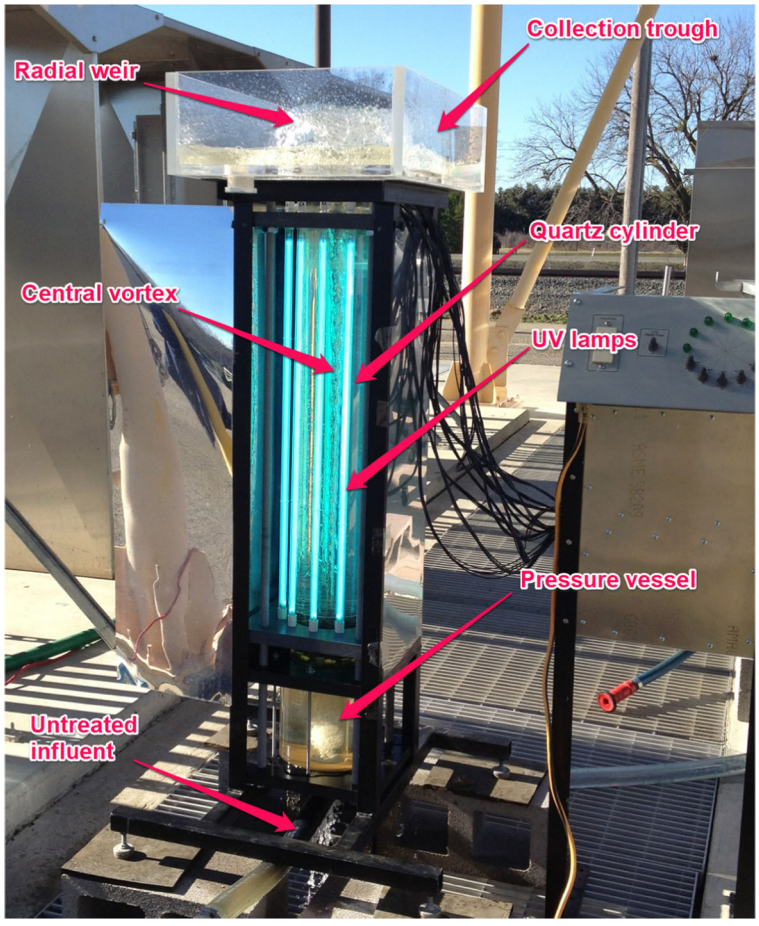

Fig. 1 Photograph of the UV system in operation

quartz cylinder; quartz being one of the few materials that allow UV-C radiation (with the 254 nm wavelength required for effective inactivation) to pass through. The manner in which the water enters this cylinder causes it to rotate as it rises to the top. The rotating motion, coupled with the presence of a drain port at the cylinder base, lead to the formation of a central air vortex in the shape of a cone with its broad base located at top of the cylinder. The UV lamps are arranged around the outside of the quartz cylinder and thus do not come in contact with the water being treated. In this way, the problem of lamp 'fouling' does not arise. The combined rotational and axial motions of the water rising induce high levels of shear stress on the inside walls of the quartz cylinder. These high levels of shear stress provide a self-cleansing mechanism in that they prevent material from adhering to the inside of the cylinder which thus remains free of 'fouling'. Due to the rotational motion of the water, the levels of turbulence kinetic energy are increased leading to vigorous mixing and hence to uniform exposure to UV radiation. Since the UV lamps are arranged circumferentially around the outside of the quartz cylinder, pathogens that may become imbedded into suspended solids experience increasing probability of receiving UV dose sufficient for their activation since the UV light is radiated from all direction. In a conventional commercial system, where the water flow is linear and parallel to the UV lamps, embedded pathogens are more likely to exit the system before receiving adequate dose. At top of the cylinder, the treated water overflows as though over a radial weir into a collection trough and from there into an outlet tube either to be re-used elsewhere. The passage of the treated water above the weir is associated with significant entrainment of air into the water thereby elevating the percentage of dissolved oxygen while simultaneously lowering the water temperature.

Testing of the UV reactor performance was carried out at the National Ornamental Research Site at the Dominican University of California (NORS-DUC). At this site, quarantine plant pathogens are studied in a mock nursery under field conditions that mimic those found in commercial nurseries (Johnson-Brousseau et al. 2011). For the experiment, irrigation water from the research site was collected and stored for 7 days prior to the experiment to have enough volume to run the reactor at a flow of 2.2 l/s and to provide the endogenous population of bacteria, oomycetes and fungi for the test. Tests were conducted on two separate occasions; in June 2013 and in October 2013. On the day of testing, this water was also spiked with *P. ramorum* zoospores to ensure its presence in the test water. *P. ramorum* zoospores were produced as described by Widmer (2009). *P. ramorum* strain 1,418,886 was grown on CV8-agar at 20 °C for approximately three weeks. Sporangia production was induced by adding 15 ml of soil extract water. Release of zoospores was induced by cold shock, and then zoospores were harvested and counted using a hemacytometer. A total of 5 l of zoospore suspension (concentration: 5×10^4 spores/ml) was added to a water tank of volume 1893 l for a final concentration of 1.3×10^2 spores/ml.

The UV reactor was placed in line with the effluent from the collection basin and was tested at a constant flow rate of 2.2 l/s. The tests were conducted under three separate conditions in which 4, 8 and 12 lamps were used. One liter of sample was collected from the influent of the disinfection system and one liter from each lamp condition to test samples for bacterial and fungal counts. The transmittance of the water was 76.4% UVT.

Bacterial counts were made from cultures growing on Reasoner's 2A, Acidified Dextrose Potato Agar (ADPA), and PARPH-V8. One milliliter of each sample was plated in triplicate on each media type and cultured using standard methods. R2A is the preferred media for culturing bacteria found in treated or potable water sources, water sources with low concentrations of endogenous bacterial populations (Reasoner and Geldreich 1985) and long incubation times (Van der Linde et al. 1999). ADPA is a media commonly used to culture fungal populations, but will also cultivate some bacterial populations. Acidification helps to reduce the amount of bacteria that will grow on the media (Mislivec and Bruce 1976). PARPH-V8 media contains pimaricin, ampicillin, rifamycin, pentachloronitrobenzene (PCNB), and hymexazol and is a selective media designed to isolate *Phytophthora sp.* (Ferguson and Jeffers 1999).

The UV dose supplied by the system was determined by quantifying its ability to remove the MS2 virus (*Escherichia coli* bacteriophage MS2 ATCC® 15597-B1™). MS2 is a male-specific (F+) RNA virus that infects bacteria. It has a similar structure to the polio virus and is widely used in water treatment research to assess the efficacy of a particular treatment method for virus removal (Bolton and Linden 2003; NWRI 2012). In the present application, water inoculated with MS2 to a given concentration was introduced into the UV

system where it was exposed to UV light produced from UV lamps that varied in number from 2 to 12. Samples of the treated water were collected and delivered on the same day to Biovir Laboratories Inc. (Benicia, CA) in accordance with the National Water Reuse Institute (NWRI) sampling guidelines (NWRI 2012). There, collimated beam testing was carried out according to standard methods (APHA 2005) to generate a dose response curve from which the actual UV dose delivered to the water was deduced. Results were obtained for water samples having UVT of 70 and 95%. The UVT was adjusted using instant coffee, an approved NWRI method (NWRI 2012). Each trial of the experiment used 1 l of MS2 with a titer of 10^{14} plaque forming units per milliliter (PFU/ml) for a final influent concentration of 10^8 PFU/ml. One liter of sample was collected for the collimated beam testing. The flow rate was kept constant at 2.2 l/s for all the tests.

Results

Analysis of bacterial inactivation

In water-treatment applications, reduction in bacterial counts achieved by a particular treatment method is measured in "log reduction" - the number of viable bacterial cells removed expressed on a logarithmic scale. For example, a 4-log reduction is a 10,000-fold decrease in the number of microorganisms present in the sample. A summary of the log reduction of the bacterial counts present in the irrigation water is presented in Table 1. Shown there are the results obtained from each of the bacterial culturing methods, and for three different lamp conditions corresponding to 4, 8 and 12 lamps. The results from the R2A and the PARPH culturing methods indicate that a minimum of 3.7 log reduction is achieved by the UV system. Low bacterial counts were seen on the APDA, because the acidification of the media suppresses bacterial growth.

Inactivation of fungi and *P. ramorum*

Microbial counts were evaluated using APDA to assess the fungal concentration and PARPH-V8 was used to determine the concentration of *P. ramorum*. Whereas true fungi (Eumycota) were present in the used nursery water as natural contaminants, *P. ramorum* was added as described above. One milliliter of each sample was plated in triplicate on each media type and cultured using standard methods. Fungal counts from the APDA media and PARPH media at each UV treatment are listed in Table 2. It can be seen there that the UV system removed about 75%, 91.7%, and 91.7% of fungal counts using 4, 8, and 12 UV lamps, respectively. It was also found that all lamp combinations of the UV system were able to inactivate 100% of the *P. ramorum* in the effluent.

Estimating the UV dose

California's water re-use policy Title 22 requires a demonstration of 5-log removal of MS-2, and the use of at least two reactors in series for redundancy to ensure a minimum level of safety in the system (NWRI 2012). Since only one reactor was tested, it must achieve at least 2.5 log removal of MS2 or a UV dose of 50 mJ/cm^2. A dose of 50 mJ/cm^2 is an approximate UV dose for 2.5 log removal. Figure 2a and b show the average log inactivation of MS2 and the average UV dose in the reactor with respect to change in lamp condition for the testing at UC Davis. From these figures, the reactor must operate with at least six lamps at a UVT of 95% and at least eight lamps at a UVT of 70% to achieve a

Table 1 Summary of bacterial reduction in irrigation water effluent

	R2A		PARPH-V8		APDA	
	Concentration, CFU/ml	Log Reduction	Concentration, CFU/ml	Log Reduction	Concentration, CFU/ml	Log Reduction
Influent	279,000 ± 19,000		48,330 ± 11,930		2330 ± 1530	
4 lamps	62 ± 3.5	3.7	4 ± 2.5	4.1	18 ± 11.9	2.1
8 Lamps	28 ± 2.8	4.0	10 ± 0.0	3.7	9 ± 0.0	2.4
12 Lamps	9 ± 2.1	4.5	1 ± 0.58	4.6	3 ± 1.7	2.9

Table 2 Summary of reduction of fungal and *P. ramorum* growth in irrigation water effluent

	APDA		PARPH-V8	
	Concentration, CFU/ml	Log Reduction	Concentration, CFU/ml	Log Reduction
Influent	4 ± 5.29		4.5	
4 lamps	1 ± 1.0	0.602	0	>0.65
8 Lamps	0.33 ± 0.58	1.08	0	>0.65
12 Lamps	0.33 ± 0.58	1.08	0	>0.65

minimum of 2.5 log removal of MS2. If only the 50 mJ/cm^2 UV dose condition is considered, then the UV reactor must operate with at least four lamps at a UVT of 95% and at least six lamps at a UVT of 70%.

Figure 2c is a plot that shows the electric energy needed by the reactor to achieve one log of MS 2 inactivation for every 3785 l of irrigation water treated. As the UVT decreases from 95 to 70%, the energy required to inactivate a log of MS2 increases as more lamps would be required to deliver the necessary UV dose. Also, for the 95% UVT case, the energy required for a log removal changes very little when more than six lamps are used. Hence this would be the ideal number of lamps that would be needed for use in these conditions.

Using the UVT of the water from NORS-DUC (76.4%), the results of testing at the Davis were used to determine the UV dose supplied to the irrigation water. Using linear interpolation with the data from Fig. 2b, the UV dose supplied to the NORS-DUC irrigation water was 51, 61, 82, 100 mJ/cm^2 for 4, 6, 8, and 12 lamps, respectively. Thus, to achieve a 5-log removal of MS2, two reactors each with four lamps would be needed to deliver the required UV dose. However, from a practical standpoint, since the percent of dissolved organics in the water can cause fluctuations in the UVT, it would be prudent to use six lamps. Achieving a 5-log removal in the irrigation water ensures that plant pathogens are not spread to the natural wildlife and renders the water suitable for reuse within the nursery.

Additional low-cost safety measures can also be used in conjunction with UV treatment to help facilitate reuse within the plant nursery, such as filtration and settling. Since UV treatment is a physical process, pathogens embedded in soil particles may be shielded from treatment (Abu-Orf et al. 2014). In fact, Title 22 requires that the turbidity of the treated water be less than 5 NTU to account for this problem (NWRI 2012). Minimizing this risk and meeting Title 22 requirements can be achieved by first allowing heavy particles to settle out of the waste stream and then screening out suspended particles with a filter before treatment, typically with a nominal diameter of 1–10 μm.

Conclusion

Treatment using ultraviolet light offers distinct advantages over chlorine and ozone because of the ease of maintenance and installation of UV systems. However, the poor transmittance and turbidity of the run-off from dissolved and suspended matter in irrigation runoff also increases the required UV dose to inactivate *Pythium* and *Phytophthora* by 2 to 4 times. The UV dose can be increased by decreasing the flow rate through the reactor, but a low flow can result in laminar flow which limits the success of the disinfection. Once flow becomes laminar, the UV dose is not equally distributed over each volume element in the reactor and active pathogens can leave the reactor (Crittenden et al. 2003). The novel UV design discussed in this paper was designed to overcome this problem and effectively treat irrigation water for harmful plant pathogens, such as *Pythium* and *Phytophthora*. In addition, this study focused on the removal of *P. ramorum* due to its particularly harmful impact on the ecosystems in many parts of the world. This study determined that the novel UV system supplies a UV dose of 51, 61, 82, 100 mJ/cm^2 when using 4, 6, 8, 12 lamps, respectively. A minimum

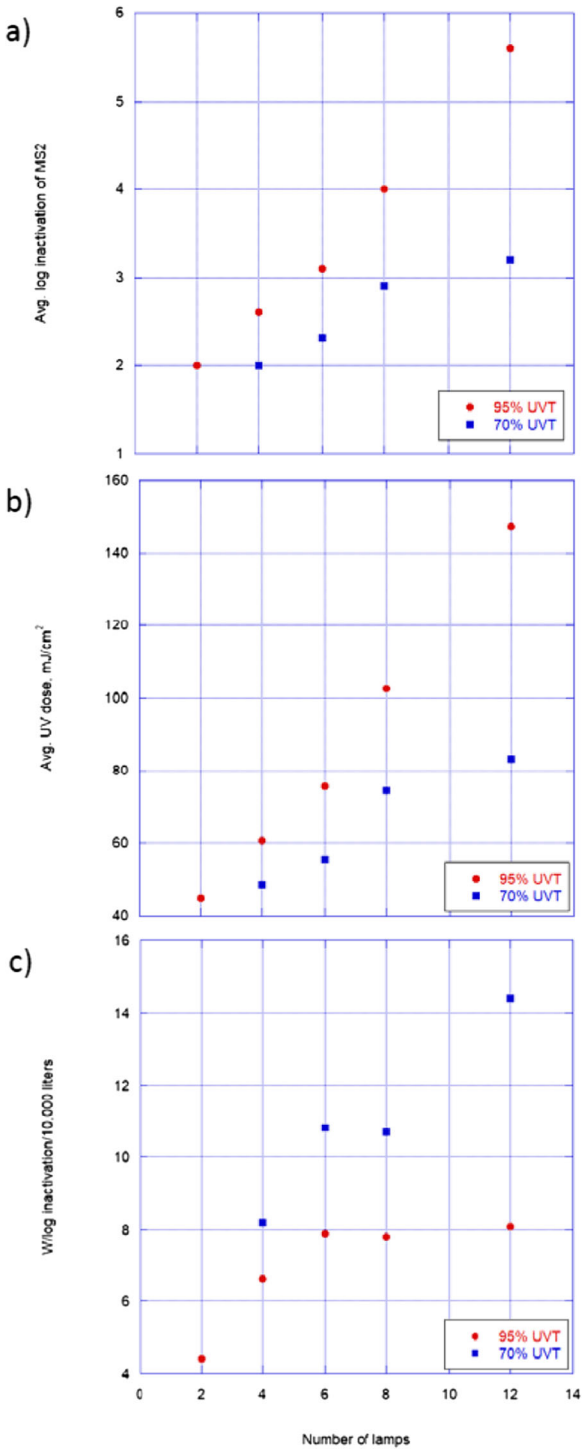

Fig. 2 Plots of the **a** average log inactivation of MS2, **b** average UV dose, and **c** energy use per log inactivation

log removal of 3.7 was obtained in the system for all lamp conditions for the removal of bacterial species. In addition, this level of treatment removed about 75%, 91.7%, and 91.7% of fungal counts using 4, 8, and 12 UV lamps, respectively. Most notably, the UV system was successful in inactivating 100% the *P. ramorum* in the influent even when only four lamps were in use.

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Compliance with ethical standards

Ethical approval The authors declare that this manuscript reports on original research that has not been published elsewhere. All the authors have read and approved this manuscript. All authors also declare that the data have not been manipulated. This manuscript does not contain any experiments with human participants or with animals.

Conflict of interest The authors declare that they have no actual or potential conflict of interest.

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