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Effect of high-power monochromatic (pulsed UV laser) and low-power broadband UV radiation on *Phytophthora* spp. in irrigation water

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Abstract Cysts or zoospores of *Phytophthora capsici*, *P. citrophthora* and *P. nicotianae* were suspended in distilled water or in recycled irrigation water collected from commercial nurseries. Propagules, suspended in shallow layers of still water in 60 mm-diameter plastic Petri dishes with lids off, were exposed to various UV doses emitted from either an excimer laser or a mercury vapor lamp. The laser emitted a monochromatic (248 nm, 5 eV/pulse), pulsed UV beam with an intensity of 1 to 2 mJ/cm^2/pulse with 20-ns pulse durations and a high peak power of 50 to 100 kW/cm^2/pulse. The UV lamp was from a commercial water purifier that emitted broadband (continuous) UV radiation at an intensity of ~8 mW/cm^2. Survival was assessed by culturing aliquots of the treated or non-treated suspensions onto corn meal agar amended with ampicillin (250 ppm) and rifampin (10 ppm), and then counting numbers of developing colonies. The UV dose (as energy per unit area) required to kill propagules was smaller when they were suspended in distilled water than when suspended in recycled nursery water. The reduced kill effectiveness in recycled water appears to be related to UV-absorbing soluble chemicals. Cysts and zoospores were equally susceptible to UV, although the high-peak power pulsed-UV laser source with ultra-short exposure times appeared to have greater kill effectiveness than the conventional Hg-vapor UV lamp. *Phytophthora capsici* and *P. nicotianae* isolates were somewhat less sensitive to UV than isolates of *P. citrophthora* obtained from various hosts and geographical regions. Furthermore, hyphae of *Phytophthora* spp. were less susceptible to UV than were cysts or zoospores.

Keywords UV disinfection · Pulsed UV · Continuous UV · Water recycling · Irrigation runoff · Water disinfection · Root disease

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Introduction

There are many reports that the introduction and spread of root infecting fungal-like organisms such as *Pythium* and *Phytophthora* spp. in fields, orchards and green houses has occurred with the use of contaminated irrigation water (Ali-Shatayeh and MacDonald 1991; Gill 1970; Grech and Rijkenberg 1992; Hong et al. 2002; Klotz et al. 1959; Kong et al. 2003; MacDonald et al. 1994; McIntosh 1966;

Mircetich et al. 1985; Oudemans 1999; Pittes and Colhoun 1984; Shokes and McCarter 1970; Thompson and Auen 1974; von Broembsen 1984; von Broembsen and Wilson 1998). The risk of pathogen spread in nursery crops is heightened when irrigation runoff water is captured and recycled. Indeed in a survey done over 18 months in northern California, large populations of *Pythium* and *Phytophthora* spp. were detected in the ponds used to capture irrigation run-off water at commercial nurseries (Ali-Shatayeh et al. 1991).

While the risk of spreading pathogens with recycled irrigation water is acknowledged, few nurseries treat their irrigation water. Instead, they rely on the use of prophylactic fungicide treatments to suppress fungal activity. However, this is not a fully effective or sustainable approach to the problem of pathogen management in irrigation water. Instead, it would be better to treat and disinfest the water before applying it to crops. Many technologies, including heat, chlorination, ozonation and UV radiation have been tested for eliminating microbes in water (Cohn and Hong 2003; Daughtry 1984; Ewart and Chrimes 1980; Hong et al. 2003; Hough 1979; Muraca et al. 1987; Parkinson 1991; Poncet et al. 2001; Stanghellini et al. 1984), However, most of the research on water treatment technologies has been focused on drinking or industrial (e.g., cooling tower) uses of water (Muraca et al. 1987; Parekh 1991; Parkinson 1991) and has been directed to the control of bacterial organisms such as *Escherichia coli* or *Legionella*. There are, however, several reports of successful treatments for fungi and other plant pathogens (Banihashemi et al. 1992; Cohn and Hong 2003; Ewart and Chrimes 1980; Grech and Rijkenberg 1992; Stanghellini et al. 1984; Yamamoto et al. 1990). Yamamoto et al. (1990) reported that ozonation of hydroponic solutions effectively reduced the number of viable spores of *Fusarium oxysporum* f. sp. *lycopersici*. Likewise Stanghellini et al. (1984) found that wide-band UV radiation controlled *Pythium aphanidermatum* in a recirculating hydroponic system used in glasshouse spinach culture .

There has been much less research on water treatments to eliminate pathogens from high-volume irrigation system. Daughtry (1984) reported using chlorine gas to treat recycled nursery water, and found it to be effective against *Pythium* and *Phytophthora*. Grech and Rijkenberg (1992) reported that electrically generated chlorine injected into citrus microirrigation systems controlled citrus gummosis and destroyed propagules of *Phytophthora nicotianae* var. *parasitica*, *P. citrophthora, Fusarium* spp., algae and slime forming bacteria, but *Tylenchulus semipenetrans* (citrus nematode) was not much affected.

Chlorination, ozonation, and ultraviolet radiation are the most widely practiced methods for killing harmful microorganisms in water (Parekh 1991). While chlorine can be effective, in commercial applications, it is most commonly applied as chlorine gas which is an extremely hazardous material. Indeed, some nurseries that formerly employed chlorine gas systems to treat their water have discontinued the use due to the difficulty of complying with environmental and worker safety regulations. Presently, most small-scale applications prefer to use a liquid chlorine injection. Ozone, while it does have some worker health-safety issues, is not as dangerous as chlorine gas and can be generated on demand, eliminating any need for storage. Ultraviolet radiation has some practical advantages over chlorination and ozonation (Adams and Robinson 1979) in that it adds no chemicals to the water (chlorination leads to gradual salt accumulation) and also can break down (through photolysis) certain pesticide residues (Peterson et al. 1990). However, unlike chlorine and ozone, UV treatment does not provide a residual, downstream disinfectant in the water which can help control slime-producing algae in distant parts of an irrigation system (Adams and Robinson 1979; Parekh 1991).

Research focusing on municipal, industrial or hydroponic solutions is not readily applicable to the issue of treating recycled irrigation water in nurseries, glass houses or field crops, where the water often flows over open ground and picks up soluble and solid impurities. These impurities can greatly degrade the efficacy of each of the treatment technologies through chemical reaction with organic impurities (in the case of chlorine and ozone) or through absorption/attenuation of UV light. The objective of the present work was to compare a commonly used wide-band, low power, continuous UV radiation source with the high powered, monochromatic pulsed UV radiation from a laser source. The killing efficacy of the two different UV sources was tested against *Phytophthora* propagules in pure water, and in recycled water collected from commercial nurseries. Preliminary results of this work were reported earlier (Banihashemi et al. 1992, 1993).

Materials and methods

Sources of isolates

Isolates of *P. capsici*, *P. citrophthora* and *P. nicotianae* used in these experiments were recovered from various hosts and locations (Table 1).

Inoculum production

Zoospores of *Phytophthora* spp. were produced by growing each species or isolates on clarified V-8 juice (CV-8) agar under fluorescent illumination at room temperature (22–24°C) for 2–4 days. Agar blocks (approximately 1-cm square) were cut and removed from the cultures, and transferred to Petri dishes containing distilled water (DW) or autoclaved soil extract (1.5 g autoclaved soil/l DW) then maintained under similar conditions for 12–24 h to allow sporangia to form. Zoospore release was triggered by moving the plates to a 4°C incubator for 20–30 min, and then returning them to room temperature. The zoospores were separated from the agar blocks by filtration through cheesecloth, yielding a zoospore suspension. For experiments involving cysts, aliquots of the zoospore suspension were vortexed to stimulate encystment. The concentration of cysts and zoospores in harvested solution were measured with a hemacytometer and adjusted as needed.

To test the effects of UV radiation on actively growing hyphae, 6-mm blocks were cut from 9–10 h old colonies growing in Petri dishes containing corn meal agar (CMA). These blocks were placed onto sterile cellophane disks that had been laid on the surface of fresh CMA in Petri dishes, or onto a glass microscopic cover slip that had been coated with a thin layer of water agar. After 4–12 h, the mycelial blocks were removed, leaving behind small patches of hyphae that adhered to the cellophane disks or the agar film on the glass cover slips. These patches of hyphae were then exposed to different UV radiation treatments.

Source of water and water treatments

Recycled irrigation water was collected fresh as needed from the recycling ponds of commercial nurseries. Most experiments were done using water collected at different times from a single, nearby nursery, but for some experiments, water samples were collected from several northern California nursery ponds. The UV attenuation in the various samples of water was measured using a Response™ scanning UV/Vis spectrophotometer (Gilford Instruments, Nova Biotech, San Diego, CA). To alter the properties of selected water samples, they were passed through filters of differing pore size; (11.6-; 1.6-; 0.6 μm pores) or diluted with various amounts of fresh tap water (1:1 or 1:3 v/v).

Broadband UV treatment

For a conventional, continuous and low-power UV source we obtained a small water purifier unit (Model 250, Ultra Dynamic Corp., Santa Monica, CA) containing a Hg lamp with an output intensity of 8 mW/cm^2 (~1 mJ/s.cm^2) with a peak emission at 254 nm. The UV lamp was mounted in a 1-cm-diameter quartz tube,

Table 1 Sources of isolates of *Phytophthora* species used in this study

Phytophthora species	Code	Source
P. capsici A_2	C-15	Bell pepper (root), CA[a]
P. capsici A_2	C-5	Squash (root), CA
P. capsici A_1	C-6	Squash (fruit), CA
P. capsici A_1	C-3	Pepper (root), NC[b]
P. capsici A_1	C-12	Chile pepper(root), CA
P. citrophthora	HAS-1696	Water (river), CA
P. citrophthora	PH-5-18-83	Pistachio (root), Iran
P. citrophthora	PH-5-15-83	Pistachio (root) Iran
P. citrophthora	P1323	Citrus(root), CA
P. citrophthora	CS	Caenothus (soil), CA
P. nicotianae	CP	Ceanothus plant (root), CA

[a] California

[b] North Carolina

positioned in the center of a 7.2-cm-diameter×38.7-cm-long stainless steel tube reactor vessel. The reactor vessel had inflow and outflow openings to allow for flowing water to be exposed.. Most of the experiments involved static water samples. This was accomplished by operating the lamp for 4–5 min to allow it to come to its full, stable UV output, and then adding 1.4 l of freshly prepared suspensions (1×10^4 propagules/ml) of zoospores or cysts, suspended either in DW or in irrigation water (IW). The reactor vessel was agitated to keep the spore suspensions moving, and 10-ml aliquots were drawn off with a pipetman at timed intervals (after 0, 5, 10, 20, or 30 s of UV exposure).

Monochromatic/pulsed UV treatment

Ten (10) ml of prepared zoospores or cysts (1×10^4 cfu/ml) suspended in DW or IW were pipetted into 6-cm-diameter plastic Petri dishes. With lids off, the plates were exposed to different UV doses from a KrF excimer laser (Lambda Physik 150 EMG Eximer Laser) that emits monochromatic UV light at 248 nm (5 eV/photon). This laser operates with UV pulses of 20 ns duration and a fluency ranging from 1–2 mJ/cm^2/pulse, which yielded a peak power of 50–100 kW/cm^2/pulse. Hyphae growing on either cellophane or cover slips were also exposed to the excimer laser beam.

Survival assessment

Before and after exposure to UV sources, the viability of zoospores and cysts was assessed by culturing 1-ml suspensions onto CMA amended with ampicillin (250 ppm) and rifampin (10 ppm) (Sigma Chemicals Pty. Ltd, UK), and counting numbers of developing colonies. Five replicate plates were used for each UV exposure. For hyphal inhibition studies, the exposed cellophane and/or cover slip containing hyphal fragments were inverted and placed onto CMA so that hyphae would be in direct contact with the agar, and the plates were observed over time to record any growth.

Statistical analysis

The experimental design for each experiment was a randomized complete block with 5 replications. All the experiments were conducted at least three times with similar and reproducible results. The data were analyzed by analysis of variance using the general linear model procedure of SAS.

Results

Sensitivity of zoospores and cysts to UV

Zoospores and cysts of *P. citrophthora, P. capsici* and *P. nicotianae* were separately suspended in distilled water and exposed to broadband, continuous, low power UV (Hg lamp). For each species, the zoospores and cysts were found to be similarly affected by UV radiation. Typical results are illustrated for *P. nicotianae* (Fig. 1), where there was a significant reduction in the numbers of viable zoospores and cysts following a 5-s exposure while virtual elimination of viable propagules resulted from 10-s or longer exposures.

Because zoospores and cysts responded similarly to UV, the experiments with monochromatic, high-peak power pulsed UV (248 nm) from the laser employed mixtures of zoospores and cysts. In these experiments, all species showed a strong dose/response effect, with propagules of *P. citrophthora* exhibiting greater sensitivity to UV than *P. nicotianae* or *P. capsici* (Fig. 2). There was 100% mortality of *P. citrophthora* propagules following exposure to 10 mJ/cm^2 UV,

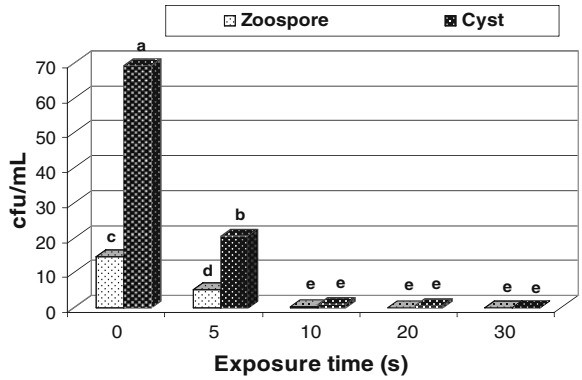

Fig. 1 Disinfection effects of low-power, continuous (Hg lamp) UV exposures on zoospores and cysts of *Phytophthora nicotianae*. The height of each bar represents the mean number of surviving cfu/ml from 3 replicate treatments. Letters have been placed over each bar to indicate significant differences. Bars labeled with identical letters indicate results that were not significantly different ($P<0.01$) according to Duncan's Multiple Range Test

while the other species required 30 mJ/cm^2 for the same effect (Fig. 2). The UV dose required for propagule mortality was actually lower than the UV dose required to prevent germination (data not shown). This was due to the fact that, except for spores exposed to very high UV doses, germination would occur, but further growth was aborted. For this reason, we determined that colony formation was a more reliable indicator of survival than simple propagule germination.

Differential sensitivity of *Phytophthora* spp. to UV

In comparison to the other two species tested, zoospores and cysts of *P. citrophthora* were more sensitive to continuous, low-power UV (data not shown) as well as to high-peak power pulsed UV (Fig. 2). We tested several additional isolates of *P. citrophthora* and *P. capsici* to determine if the difference was species- or isolate-related. These experiments involved isolates of each species from different geographic locations and hosts (Table 1). Samples of zoospores of the *P. citrophthora* and *P. capsici* isolates were suspended separately in DW and exposed to various UV doses of the high-peak power pulsed UV laser source. While the different isolates yielded different initial numbers of zoospores, all isolates of *P. capsici* required >20 mJ/cm^2 for nearly complete mortality (Fig. 3), while all isolates of *P. citrophthora* were killed at nearly half that UV dose (Fig. 4).

While the zoospores and cysts of each *Phytopthora* species tested were killed by exposure to UV doses of 15–30 mJ/cm^2, depending upon species, actively-growing mycelium was much less sensitive. UV doses up to 40 mJ/cm^2 had no discernable effect on the growing hyphae, although none survived exposures to 60 mJ/cm^2 (data not shown).

Efficacy of UV in recycled irrigation water

In initial experiments, we found that when zoospores and cysts of *P. citrophthora* and *P. capsici* were suspended in recycled irrigation water, it required two to four times as much UV energy to cause mortality as compared to propagules suspended in DW (data not shown). We attributed this difference to the absorption of UV energy by organic contaminants and suspended solids present in recycled irrigation water. For example, the depth of penetration (in cm) of 248-nm UV photons from the high-power pulsed UV laser source was reduced by ≥50% by a variety of recycled water samples from several nursery locations, as compared with fresh irrigation district water (Fig. 5). Experiments comparing the efficacy of continuous, low-power conventional UV (Hg lamp) against *P. capsici* zoospores suspended in DW, raw recycled irrigation water (RW), or recycled irrigation water filtered to remove particulates >0.6 mm, demonstrated that the attenuation of UV energy was caused by both suspended solid and soluble impurities (Fig. 6).

Finally, diluting recycled irrigation water with fresh water greatly improved the pulsed UV (248 nm) penetration (Fig. 7). This approach could be used in

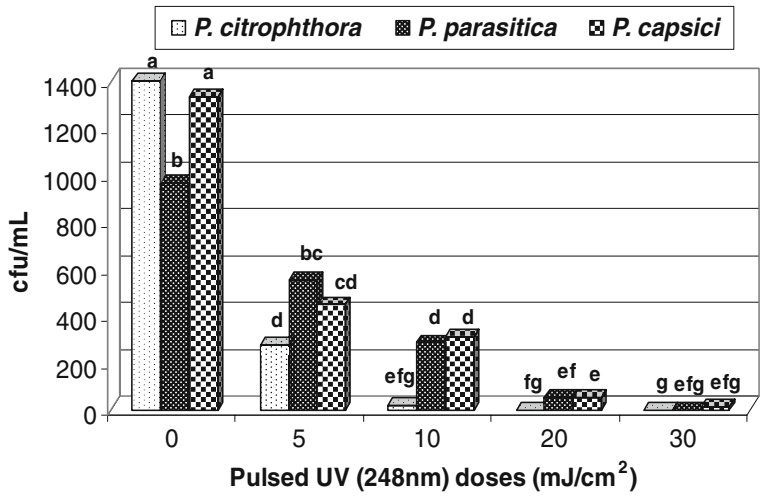

Fig. 2 Disinfection effects of high-peak power pulsed UV (248 nm) laser radiation on mixtures of zoospores and cysts of *Phytophthora nicotianae*, *Phytophthora citrophthora* and *Phytophthora capsici*. The height of each bar represents the mean number of surviving cfu/ml from 3 replicate treatments. Letters have been placed over each bar to indicate significant differences. Bars labeled with identical letters indicate results that were not significantly different ($P<0.01$) according to Duncan's Multiple Range Test

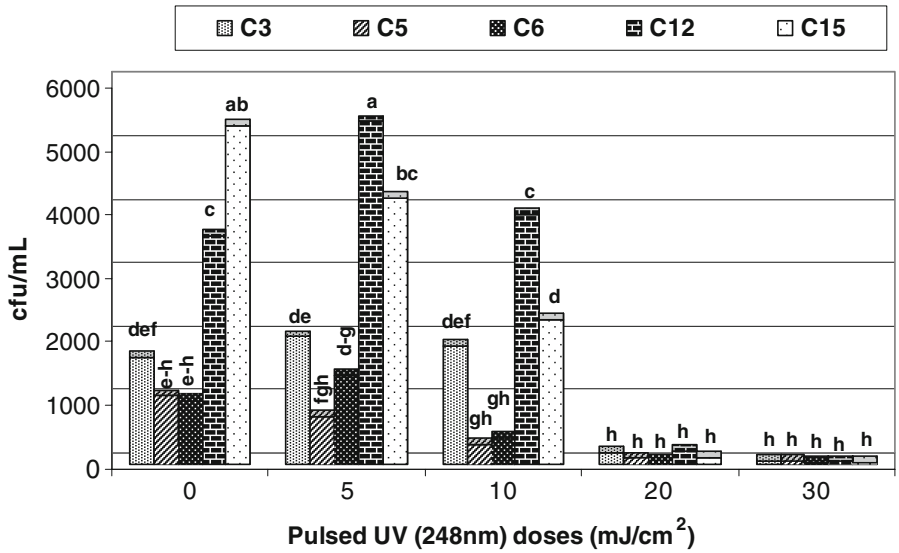

Fig. 3 Differential sensitivity of zoospores of different isolates of *Phytophthora capsici* (see Table 1) to high peak power pulsed UV (248 nm) laser radiation. The height of each bar represents the mean number of surviving cfu/ml from 3 replicate treatments. Letters have been placed over each bar to indicate significant differences. Bars labeled with identical letters indicate results that were not significantly different ($P<0.01$) according to Duncan's Multiple Range Test

nursery operations. In experiments in which zoospores of *P. capsici* were suspended in DW, RW, or RW diluted with fresh district irrigation water, showed that dilutions of 1:1 greatly enhanced efficacy of pulsed UV (248 nm) radiation induced disinfection (Fig. 8).

Discussion

The ability to reliably disinfect irrigation water so that it does not contribute to pathogen spread in crops is a matter of great concern to nursery growers especially in those operations based upon recycled irrigation water. Various methods have been adopted to treat irrigation water (Adams and Robinson 1979; Cohn and Hong 2003; Daughtry 1984; Ewart and Chrimes 1980; Grech and Rijkenberg 1992; Hong et al. 2003; Poncet et al. 2001; Stanghellini et al. 1984), but there is a continuing need to better understand how these methods are influenced by the contaminants that are typically present at concentration levels that fluctuate widely through the growing seasons.

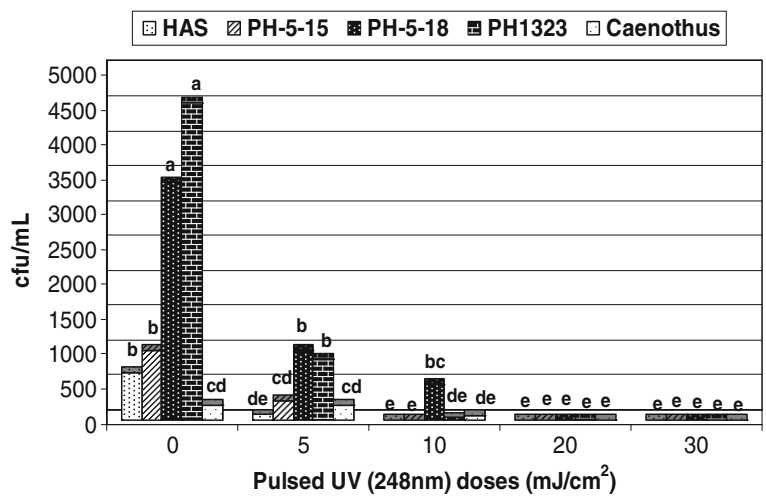

Fig. 4 Differential sensitivity of zoospores of different isolates of *Phytophthora citrophthora* to high-peak power, pulsed UV (248 nm) laser radiation. The height of each bar represents the mean number of surviving cfu/ml from 3 replicate treatments. Letters have been placed over each bar to indicate significant differences. Bars labeled with identical letters indicate results that were not significantly different ($P<0.01$) according to Duncan's Multiplae Range Test

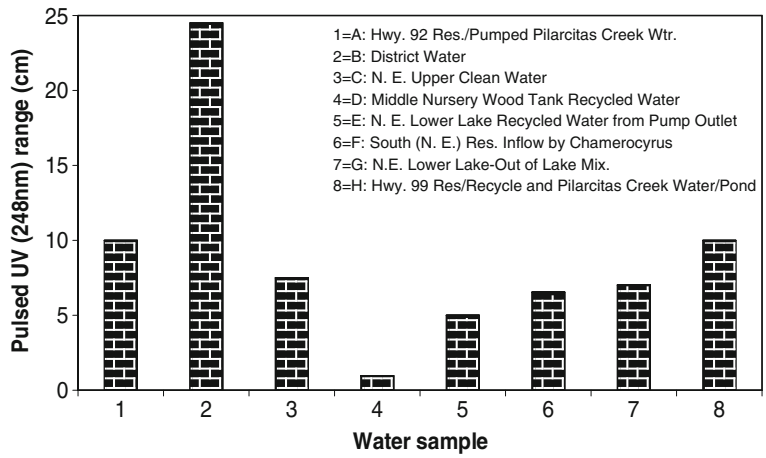

Fig. 5 Range (depth) of penetration (cm) of UV radiation in different sources of irrigation water. Range of penetration through water was measured as the thickness of a water layer that yielded a 100 times (2 $\log_{10}$) reduction in UV radiation intensity from a pulsedUV (248 nm) laser source

Among the common disinfection options (e.g., chlorine, ozone, continuous UV), Hg-lamp UV technology has enjoyed some popularity because it is relatively easy to install, maintain, and presents few worker safety risks. While it does not provide the residual, downstream disinfection benefits of cholorine and, to a lesser extent with ozone, it can aid in the breakdown of organic pesticide residues in water (Peterson et al. 1990). Besides, while the efficacy of UV disinfection is degraded by contaminants in the water, including materials of a near-colloidal, if not soluble nature (Fig. 6), efficacy of chlorine and ozone disinfection is also degraded by the same contaminants reacting and consuming the disinfectant chemicals and reducing their concentrations due to secondary chlorination or ozonation reactions. In practice, increasing the initial concentration of chlorine or ozone applied to disinfest irrigation water can compensate for these losses but increases costs and operating risks.

On the other hand, the loss in UV disinfection efficacy between clear water and recycled irrigation water is also substantial (Fig. 6). However, overcoming this effect in a conventional Hg-lamp UV system can be accomplished by reducing the flow rate to increase UV exposure time. Nevertheless, this simple option can greatly reduce throughput capacity and is,

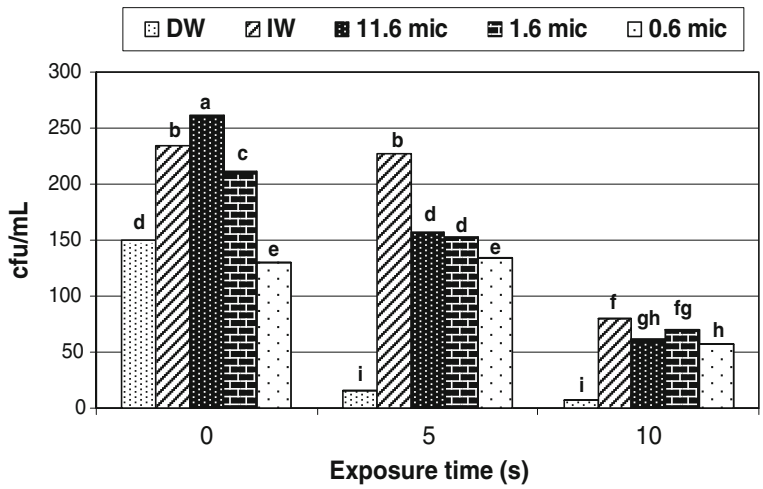

Fig. 6 Comparative UV disinfection of water containing zoospores of *Phytophthora capsici* and exposed to a low-power, continuous (Hg lamp) UV radiation source. Spores were suspended in distilled water (DW), recycled irrigation water (IW), and irrigation water passed through different pore-size filters (11.6, 1.6 and 0.6 micrometers). The height of each bar represents the mean number of surviving cfu/ml from 3 replicate treatments. Letters have been placed over each bar to indicate significant differences. Bars labeled with identical letters indicate results that were not significantly different ($P<$ 0.01) according to Duncan's Multiple Range Test

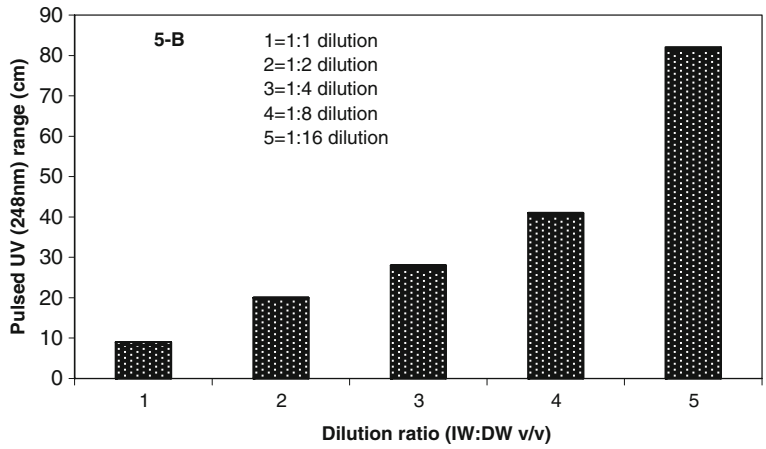

Fig. 7 Effects of dilution (v/v) on extending range (depth) of penetration of UV radiation in irrigation water. Range of penetration was measured as the thickness of a water layer that yielded a 100 times (2 $\log_{10}$) reduction in UV radiation intensity from a pulsedUV (248 nm) laser source

in practice, not fully effective. In experiments at a commercial nursery using our small UV water purifier (data not shown) we found that reducing the flow rate through the reactor vessel did increase propagule mortality, but that at very slow flow rates (i.e., longer UV exposure times), there still was a fraction of the population of propagules that remained viable. This was attributed to the establishment of laminar flow conditions at very low flow rates through the reactor vessel such that some propagules near the walls of the reactor vessel never were close enough to the light source to absorb a lethal UV dose. This attenuation effect could be overcome by improved reactor vessel design to include adequately shaped baffles to ensure turbulent flow even at low flow rates. It is expected that the same conditions would exists in either the low-power, continuous Hg-lamp UV technology or in the high-peak power pulsed UV systems.

Another strategy to improve efficacy with any of the technologies mentioned here but particularly beneficial to the UV disinfection options is to dilute the recycled irrigation water with fresh water. Our UV experiments showed that dilution with fresh water significantly enhanced the efficacy of UV treatment (Fig. 8).

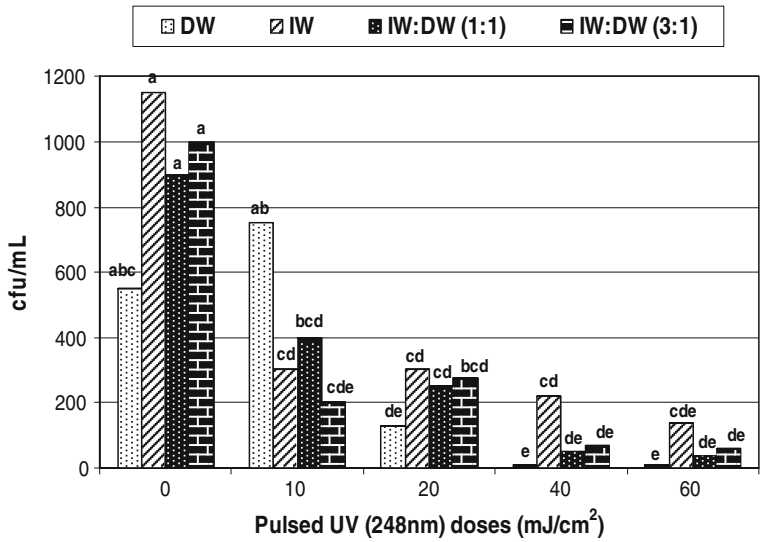

Fig. 8 Effects of dilution (v/v) of recycled irrigation water (IW) with fresh water on UV disinfection efficacy of *Phytophthora capsici* with high-power, pulsed UV (248 nm) laser radiation. The height of each bar represents the mean number of surviving cfu/ml from 3 replicate treatments. Letters have been placed over each bar to indicate significant differences. Bars labeled with identical letters indicate results that were not significantly different ($P<0.01$) according to Duncan's Multiple Range Test

Yet another novel and emerging approach for enhanced UV disinfection efficacy is to utilze a more powerful source of high-peak power pulsed UV radiation. In the comparison experiments using the low-power, continuous Hg lamp UV source, between 5–10 s exposures were required to achieve complete mortality of *P. citrophthora* spores in DW (Fig. 1). However, if a pulsed UV (248 nm) laser source is used, a dose of just more than 10 mJ/cm^2 was required for complete mortality of *P. citrophthora* spores (Fig. 4). Because the pulsed UV laser source emitted 1–2 mJ/cm^2 per each 20-ns duration pulse, a 4 pulse-per-second (pps) repetition rate was capable of delivering a lethal UV dose in only 2–4 s exposures. As new arc or flashlamp technologies are now available operating with 10 pps repetition rates and emitting ~400 J/pulse (1 ms duration) commercial systems based on these technologies could allow for much greater throughput capacity than conventional UV standard Hg lamps and provide reliable operational capabilities and cost benefits when compared with current chemical and standard UV options (Lagunas-Solar 2005; Lagunas-Solar et al. 2009).

Results of this work confirmed that UV radiation is effective to inactivate propagules of *Phytophthora* spp. in recycled irrigation water. However, the successful application of emergent pulsed UV technologies in the nursery environment may necessitate some improvements in reactor vessel design to prevent laminar flow at various operating flow rates while some changes in irrigation water management (e.g., to allow dilution of recycled water prior to treatment) may need to be evaluated.. Finally, it was proved that the use of the more powerful sources of pulsed UV offer great promise for water treatment and deserves to be fully investigated.

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