

*Short Communication*

# Antimicrobial Efficacy of Aqueous Ozone in Endodontic Root Canals

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Aqueous ozone ( $O_{3(aq)}$ ) solutions were applied to the rockwool substrate of hydroponically cultured tomato and cucumber plants. Single applications of high concentration solutions (0, 5, 10, 15, 20 mg/L), as well as repeated application of lower concentration solutions (0, 2, 4, 6 mg/L), had no impact on leaf area and shoot dry weight accumulation. Repeated  $O_{3(aq)}$  applications were also applied to cucumber plants inoculated with *Pythium aphanidermatum*. Pathogen levels were significantly reduced in all treatments containing  $O_{3(aq)}$ . The reduction in pathogen numbers did not necessarily affect plant productivity.

**Key words:** *Pythium aphanidermatum*, irrigation water reuse, oxygenation, tomato, cucumber, rockwool, hydroponics, phytotoxicity.

## INTRODUCTION

In nearly all of the world's major greenhouse and nursery production regions, water is now the limiting resource. Managers face water supply challenges in the form of restrictions, competing uses, deteriorating quality (e.g. salinity, chemical contamination etc.), and rising costs associated with accessing reliable supplies (Bouwer, 2000). These challenges have fostered a shift towards the collection and reapplication of irrigation waters (Bouwer, 2000; Richard et al., 2006). Although this makes good use of a limited resource, it contributes to a second major production challenge in greenhouse and nursery systems, namely disease proliferation.

In absence of a system to treat the recovered water, growers risk disease proliferation via the reapplication of contaminated solutions. Many options are available for treating the recovered solutions, including filtration, heat, surfactants, ultraviolet radiation, and chemical disinfection (Cayanan et al., 2008; Ehret et al., 2001). Aqueous ozone ( $O_{3(aq)}$ ) is also an option in some greenhouse and nursery settings (Ehret et al., 2001; Graham et al., 2009), as it is a proven water disinfection technology with over 100 years of application experience from which to draw.

Although a proven technology, widespread adoption of  $O_{3(aq)}$  as an irrigation water remediation tool has been slow due to actual and perceived limitations. The first

limitation is the cost and complexity of the systems, which currently limits the use of ozone to larger operations. This being said, continual advances in ozone generation and dissolution technologies may soon address these barriers. A second major limitation is the fact that ozone is a known phytotoxic gas. This phytotoxicity has been clearly demonstrated by many studies over the past 50 years that have examined plant responses to troposphere ozone enrichment (Bell and Treshow, 2002).

Although gaseous ozone can be phytotoxic at low concentrations (Bergmann et al., 1999), in aqueous solution, the mass transfer physics and chemical stability are much different than in the free gas state (Gottschalk et al., 2000). This difference is often overlooked when developing treatment applications for irrigation systems, thus hindering the development of alternative disease management protocols that do not suffer from the afflictions of standard commercial pest control strategies. Unlike commercial pesticides,  $O_{3(aq)}$  does not leave a residual nor is the development of pathogen resistance likely as ozone reacts with diverse cellular constituents (Guzel-Seydim, 2004).

Ozone is unstable in solution; any ozone that has not reacted with chemical or biological contaminants reverts to diatomic oxygen (Beltrán, 2004), which in itself has potential for improving crop performance (Zheng, 2007; Drew, 1997). Growers incorporating ozone into their irrigation management strategy typically allow the ozone to dissipate or actively remove it prior to distribution to the

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crop. This removal is carried out as a prudent action to avoid any potential crop damage resulting from ozone off-gas. This prudence is particularly justified in overhead irrigation systems where significant off-gassing can occur, which if not properly managed can cause foliar damage (Graham et al., 2009). When applied directly to the growth substrate (e.g. drip) this risk is greatly reduced, as the solutions are not exposed to the bulk atmosphere. The little information that is available regarding the direct application of  $O_{3(aq)}$  to growth substrate, suggests that the phytotoxic potential may be overestimated and the use of  $O_{3(aq)}$  may hold promise for diversifying irrigation management options (Ohashi-Kaneko et al., 2009; Sloan and Engelke, 2005).

## EXPERIMENTATION

Experiments were conducted to develop an initial understanding of the potential for using  $O_{3(aq)}$  solutions as a component of a greenhouse or nursery irrigation management plan. Tomato (*Solanum lycopersicum* L. cv Trust F1) and cucumber (*Cucumis sativus* L. cv. Serenade F1) plants were grown in rockwool hydroponic culture and subjected to  $O_{3(aq)}$  irrigation regimes in isolation or in combination with a pathogen (*Pythium aphanidermatum*) challenge. The objectives were: (1) to determine if  $O_{3(aq)}$  applied directly to a rockwool hydro-ponic substrate suppressed productivity (as measured by leaf area and dry matter accumulation); and (2) determine if  $O_{3(aq)}$  applied directly to a rockwool growth substrate can reduce the incidence of *P. aphanidermatum*.

## RESULTS AND DISCUSSION

Although these studies are limited in scope, there was clear indication that aqueous ozone could be applied directly to the surface of rockwool growth substrate in both tomato and cucumber hydroponic culture without adversely influencing growth (Figure 1). It was also evident that some level of pathogen suppression was achieved through the application of  $O_{3(aq)}$ , although the connection between reduced pathogen presence and the maintenance of plant performance was not definitive (Figure 2).

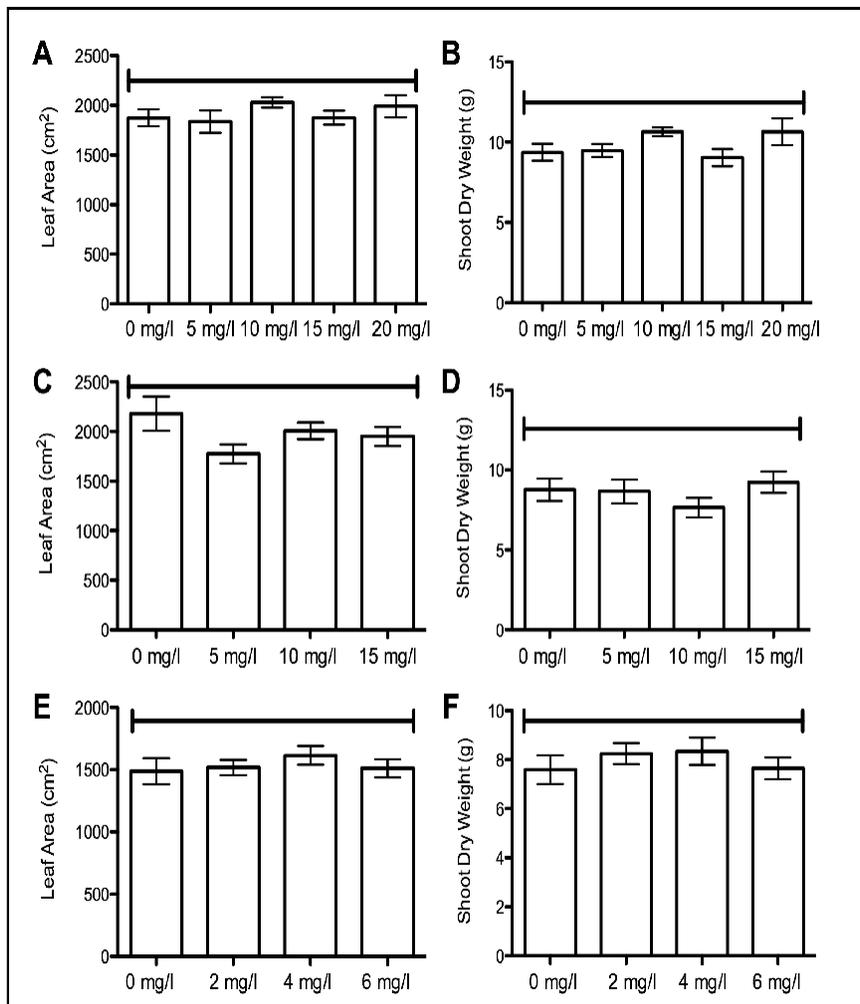
In the first two studies, 2 L aliquots of solutions containing high  $O_{3(aq)}$  concentrations (5, 10, 15, 20 mg/L) were applied in a single dose to the root zones of tomato and cucumber plants. The results (Figures 1A-D) clearly showed that there were no discernible effects on growth as determined by the leaf area and dry matter accumulation. These results were somewhat unexpected as the concentrations employed were excessive in comparison to typical water treatment applications. These same concentrations, when applied as a foliar drench, elicit varying degrees of phytotoxicity (data not shown) (Graham et al., 2009). During treatment application, the drainage was collected and the ozone residual was measured. In all cases, very low (<0.03 mg/L) or no ozone remained in the solution after passage through the rockwool block (as

measured by standard indigo methods, (Hach Co. Loveland CO, USA)). This was an indication that the majority of ozone reacted with some component of the root – substrate complex (including micro-organisms, and chemical constituents). Visual examination of the root mass revealed no symptomatic evidence of root browning or other damage indicative of oxidative stress.

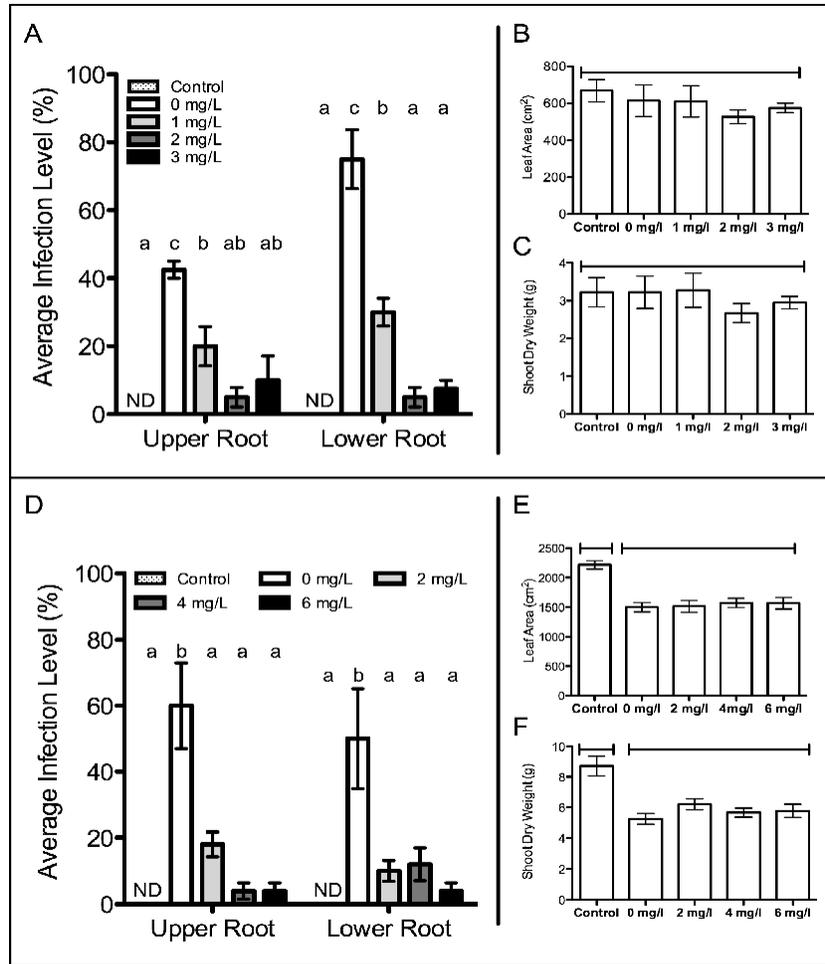
Transient applications, even at the very high concentrations employed in the first two studies, may not have been sufficient to cause any visible phytotoxicity symptoms. It was theorized that a mature plant with an established root zone ecosystem would have a significant buffer (against ozone damage) in the form of accumulated organic compounds and micro-organisms. Repeated applications may overwhelm this buffer capacity. In a third study, the concentrations applied were reduced to 0, 2, 4, 6 mg/L  $O_{3(aq)}$  but were applied twice daily for six days. Once again, the results (Figures 1E-F) indicated that there was no loss in production even with these frequent ozone applications. Recent work by Ohashi-Kaneko et al. (2009), in which ozone was applied to tomato plants once per week for three weeks at 1.5 mg/L, supports these findings.

Given that no phytotoxic responses were observed during these studies and combined with the potential for improved productivity shown by others (Ohashi-Kaneko et al., 2009; Sloan and Engelke, 2005), the question is then one of disease control benefits. If  $O_{3(aq)}$  does not cause any significant negative growth responses then it may have potential to be used in the control of disease vectors in the root zone (McDonald, 2007). In a third series of short experiments, cucumber plants were inoculated with *P. aphanidermatum* and subjected to a series of ozone treatments. The results are summarized in Figure 2.

Although the application of  $O_{3(aq)}$  clearly reduced the presence of *P. aphanidermatum* in both studies (Figures 2A and D), it did not necessarily result in a maintenance of productivity under intense disease pressure (Figures 2E-F). Given the limited scope and the failure for the studies to corroborate one another in terms of productivity maintenance (Figures 2B-C, E-F), only limited inferences can be made on the efficacy of  $O_{3(aq)}$  to prevent production loss due to disease. The pathogen load was clearly reduced in both studies (Figures 2A and D), which suggests that ozone is reaching the roots; however, the treated plants in the higher dose study did not maintain the production levels observed in the control plants. In this case,  $O_{3(aq)}$  was either ineffective against the establishment of the pathogen (as inferred from SDW and LA data), or the combination of biotic and abiotic stressors (disease and high  $O_{3(aq)}$ ) acted to suppress productivity. This is not to say that disease control and maximum productivity could not be achieved under a more rigorous treatment protocol, as evidenced in Figures 2A-C, but rather that the complexities of the systems should not be underestimated.



**Figure 1.** Leaf area (LA) and shoot dry weight (SDW) response of tomato and cucumber to direct applications of aqueous ozone to the rockwool (Grodan Delta-10 Gro-blocks 10x10x 6.5cm) growth substrate: A-B) Response of six week old tomato plants to a one time root zone  $O_{3(aq)}$  application. Each plant received a 2 litre aliquot from one of five  $O_{3(aq)}$  solutions (0, 5, 10 15, 20 mg/l). The solutions were poured over the rockwool cube at an average rate of 1 l/min. Plants were grown for an additional 12 days before being destructively analysed (n=6); C-D) Response of six week old cucumber plants to a one time root zone  $O_{3(aq)}$  application. Each plant received a 2 litre aliquot from one of four  $O_{3(aq)}$  solutions (0, 5, 10 15 mg/l). The solutions were poured over the rockwool cube at an average rate of 1 l/min. Plants were grown for an additional 10 days before being destructively analysed (n=5); E-F) Response of six week old tomato plants to twice daily (10:00 and 17:00) root zone applications of a 1 litre  $O_{3(aq)}$  solution (0, 2, 4, 6 mg/l) . Treatments commenced when the plants were six weeks old and continued for 6 days, after which the plants were grown for an additional 7 days before being destructively analysed: A-F) Columns falling under the same horizontal line are not statistically different at  $p < 0.05$ ; error bars are  $\pm$  standard error of the mean (one-way ANOVA with Tukey's post test, GraphPad Prism ver. 5.0c for Mac, GraphPad Software, San Diego, Calif. USA). Ozone solutions were prepared using an oxygen-fed (90-95%  $O_2$ ) corona discharge ozone generator (CD1500P, Clearwater Tech., San Luis Obispo, CA., USA) and a Shaw Mixer<sup>TM</sup> ozone mass transfer system (Purification Research Technologies Incorporated, Guelph, Ontario, Canada). Ozone concentrations were measured with a dissolved ozone sensor (Q45H, ATI, Collegeville, PA, USA) calibrated against the indigo method (Bader and Hoigne, 1981) . LA was determined using a leaf area meter (LI-3100C, Li-Cor, Lincoln, NE). SDW was determined after drying all samples to a constant mass. All plants were grown in a research greenhouse at the University of Guelph.



**Figure 2.** Response of cucumber plants grown in rockwool growth media (Grodan Delta-10 Gro- blocks 10x10x 6.5cm), previously inoculated with *P. aphanidermatum*, to application of  $O_{3(aq)}$ : A) Average infection level (percent of root segments sampled) in the upper and lower half of the root mass for treatments consisting of a Control (not inoculated, no ozone), 0, 1, 2, 3 mg/l  $O_{3(aq)}$ . The plants were inoculated and allowed to stand for several hours before the first treatment application. One litre aliquots (per plant) were applied to the rockwool substrate twice daily (1 l/min; 10:00 and 17:00) for 14 days (n=4); B-C) Leaf area and shoot dry weight response to the treatments described in A.; D) Average infection level (percent of root segments sampled) in the upper and lower half of the root mass for treatments consisting of a Control (not inoculated, no ozone), 0, 2, 4, 6 mg/l  $O_{3(aq)}$ . The plants were inoculated and allowed to stand for several hours before the first treatment application. One litre aliquots (per plant) were applied to the rockwool substrate twice daily (1 l/min; 10:00 and 17:00) for 14 days (n=5); E-F) Leaf area and shoot dry weight response to the treatments described in D; A-F) Columns falling under the same horizontal line or those having the same letter appearing above it as other columns in the group are not significantly different at  $p < 0.05$ ; error bars are  $\pm$  standard error of the mean (one-way ANOVA with Tukey's post test, GraphPad Prism ver. 5.0c for Mac, GraphPad Software, San Diego, Calif. USA). Ozone solutions were prepared using an oxygen-fed (90- 95%  $O_2$ ) corona discharge ozone generator (CD1500P, Clearwater Tech., San Luis Obispo, CA., USA) and a Shaw Mixer<sup>TM</sup> ozone mass transfer system (Purification Research Technologies Incorporated, Guelph, Ontario, Canada) . Ozone concentrations were measured with a dissolved ozone sensor (Q45H, ATI, Collegeville, PA, USA) calibrated against the indigo method (Bader and Hoigne, 1981) . LA was determined using a leaf area meter (LI-3100C, Li-Cor, Lincon, NE). SDW was determined after drying all samples to a constant mass. All plants were grown in a research greenhouse at the University of Guelph. *Pythium aphanidermatum* inoculum was prepared by selection on P5 media followed by a propagation phase in V8 media, which was then applied as a root drench. ND - Pathogen Not Detected.

Given these results, it is reasonable to assume that routine application of  $O_{3(aq)}$  could prevent the establishment of pathogens in rockwool hydroponic culture. In the presented studies, the plants were specifically inoculated with a rich source of *P. aphanidermatum* to elicit a disease response. In an actual production system such a deliberate and strong disease exposure would likely be rare. Frequent applications of  $O_{3(aq)}$  could therefore have some potential for disease prevention in commercial settings; a potential that warrants further investigation.

The results presented justify a more thorough evaluation of the direct application of  $O_{3(aq)}$  to the growth substrate of common greenhouse and nursery crops. Focus should be given to the determination of concentration and application frequency thresholds as well as investigations into the influence of substrate type and application timing (to take advantage of diurnal stomatal states). Clearly the phytotoxicity dynamics of ozone in aqueous solution is different than that of the gas phase. This knowledge opens the door to a range of horticultural  $O_{3(aq)}$  applications related (primarily) to irrigation system maintenance and pest management.

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