New Perspectives on the Maintenance of Aqueous Ozone Residuals in Greenhouse and Nursery Irrigation Solutions

by

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ABSTRACT

New Perspectives on the Maintenance of Aqueous Ozone Residuals in Greenhouse and Nursery Irrigation Solutions

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Ozonation has been utilized for water treatment for over 100 years. During that time, the range of applications has grown considerably, and includes the remediation of nursery and greenhouse irrigation water. Ozone is dissolved into irrigation water to kill pathogens and degrade chemical contaminants. By convention, growers remove ozone from solutions, prior to distribution to the crop, to avoid phytotoxic effects. The available literature regarding aqueous ozone (O$_3$(aq)) phytotoxicity is limited, making this a sagacious practice, although the removal does preclude any ancillary benefits beyond the point of treatment.

The effects of applying O$_3$(aq) under two irrigation systems are examined. Initial studies suggested O$_3$(aq) concentrations as high as 20 mg L$^{-1}$ could be applied directly to mineral wool substrate in a limited (one time) fashion without a negative response. To be effective as a remediation tool, however, ozone would need to be applied more frequently (e.g. daily). The effects of daily O$_3$(aq) application, via drip irrigation in mineral wool hydroponic tomato culture, was examined. In the first of two studies, daily applications of 3.0 mg L$^{-1}$ O$_3$(aq) elicited an overall positive growth response. In a follow-up study, 6.0 mg L$^{-1}$ O$_3$(aq) elicited a negative response.

Nursery operators often utilize overhead irrigation. A study was conducted to determine if overhead irrigation utilizing O$_3$(aq) was compatible with select woody perennial nursery species. The amount of ozone lost from solution during application was examined, as well as crop response to the ozone environment generated. It was shown that 60 to 70% of the ozone was unaccounted for at canopy level, while phytotoxic effects were elicited at emitter concentrations above 1.5 mg L$^{-1}$.

Marchantia polymorpha is a significant weed species in greenhouse and nursery production; a species with few control options. Anatomical features of M. polymorpha suggested sensitivity to O$_3$(aq).
Studies were performed to examine contact time (CT) and exposure frequencies required for *M. polymorpha* suppression. A CT of 0.84 mg·L\(^{-1}\)·min at an application frequency of 3-times/week achieved measurable suppression.
DEDICATION

To my loving wife Cindy, my Mom and Dad, and ‘What Will They Think of Next?’

You inspired me.
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‘No man is an island’, and nowhere in my professional career has this been more apparent than during the years spent working towards this degree. I would not have been able to complete this work without the support, encouragement, and assistance from a great number of family, friends, and colleagues. There are too many of you to thank without having to add another chapter to this beast, but you all know who you are and what you have done and meant to me over the years. Having said this, there are a few that really need to be singled out (no particular order):

Ping Zhang: 砰地作聲，您是不僅我的右手，但是經常我的左手。我永遠是在您的負債。I hope the translation is close, but if not here is what I really was trying to say: Ping, you were not only my right hand but often my left hand as well. I am forever in your debt.

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Fig. A1.2: Two-film mass transfer model in a water droplet system. The layers depicted in the model presented in Fig. 1 are present in concentric spheres. To account for the spherical geometry, the surface area : volume ratio for the drop is incorporated into the specific mass transfer rate. The standard concentration gradient is shown in red; however, for the calculations presented the gas phase is assumed to be uniform (i.e. single film layer).

Fig. A2.1: Leaf area (LA) and shoot dry weight (SDW) response of tomato and cucumber to direct applications of aqueous ozone to the mineral wool (Grodan Delta-10 Gro-blocks 10cm x 10cm x 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 L aliquot from one of five $O_{3(aq)}$ solutions (0, 5, 10, 15, 20 mg·L$^{-1}$). The solutions were poured over the mineral wool cube at an average rate of 1 L·min$^{-1}$. Plants were grown for an additional 12 d 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 L aliquot from one of four $O_{3(aq)}$ solutions (0, 5, 10, 15 mg·L$^{-1}$). The solutions were poured over the mineral wool cube at an average rate of 1 L·min$^{-1}$. Plants were grown for an additional 10 d 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 L $O_{3(aq)}$ solution (0, 2, 4, 6 mg·L$^{-1}$). Treatments commenced when the plants were six weeks old and continued for 6 days, after which the plants were grown for an additional 7 d before being destructively analysed: A-F) Columns falling under the same horizontal line are not statistically different at P<0.05; error bars are +/- SE 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™ 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.
determined after drying all samples to a constant mass. All plants were grown in a research greenhouse at the University of Guelph and were 42 d old at harvest. *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 – disease symptoms not detected........................................................................................................................................151
# List of Abbreviations Symbols & Nomenclature

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Carbon Dioxide Assimilation rate</td>
</tr>
<tr>
<td>Aqueous Ozone</td>
<td>Water with a residual ozone concentration <em>still present</em></td>
</tr>
<tr>
<td>c_i</td>
<td>Intercellular Carbon Dioxide Concentration</td>
</tr>
<tr>
<td>95%CI</td>
<td>95% Confidence Interval</td>
</tr>
<tr>
<td>CORD</td>
<td>Canada-Ontario Research and Development Program</td>
</tr>
<tr>
<td>CRD</td>
<td>Completely Randomized Design</td>
</tr>
<tr>
<td>CT</td>
<td>Contact Time</td>
</tr>
<tr>
<td>DAQ</td>
<td>Data Acquisition</td>
</tr>
<tr>
<td>DBP</td>
<td>Disinfection By-Products</td>
</tr>
<tr>
<td>DI</td>
<td>Deionized Water</td>
</tr>
<tr>
<td>DTPA</td>
<td>Diethylene triamine pentaacetic acid</td>
</tr>
<tr>
<td>EC</td>
<td>Electrical Conductivity</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>Fertigation</td>
<td>Delivery of nutrients/fertilizer via the irrigation solution</td>
</tr>
<tr>
<td>GIMP</td>
<td>GNU Image Manipulation Program</td>
</tr>
<tr>
<td>g_s</td>
<td>Stomatal Conductance</td>
</tr>
<tr>
<td>hr</td>
<td>Hours</td>
</tr>
<tr>
<td>ISM</td>
<td>Integrated Solution Management</td>
</tr>
<tr>
<td>LA</td>
<td>Leaf Area</td>
</tr>
<tr>
<td>LDI</td>
<td>Leaf Damage Index</td>
</tr>
<tr>
<td>Liverwort</td>
<td><em>Marchantia polymorpha</em></td>
</tr>
<tr>
<td>LOX</td>
<td>Liquid Oxygen</td>
</tr>
<tr>
<td>min</td>
<td>Minutes</td>
</tr>
<tr>
<td>OCE</td>
<td>Ontario Centres of Excellence</td>
</tr>
<tr>
<td>OMAFRA</td>
<td>Ontario Ministry of Agriculture, Food and Rural Affairs</td>
</tr>
<tr>
<td>O_3(aq)</td>
<td>Aqueous ozone formula</td>
</tr>
<tr>
<td>O_3(g)</td>
<td>Gaseous ozone formula</td>
</tr>
<tr>
<td>OSH</td>
<td>Occupational Health and Safety</td>
</tr>
<tr>
<td>ppb</td>
<td>Parts per billion</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
</tr>
<tr>
<td>PSA</td>
<td>Pressure Swing Adsorption</td>
</tr>
<tr>
<td>PRTI</td>
<td>Purification Research Technologies Incorporated</td>
</tr>
<tr>
<td>RCBD</td>
<td>Randomized Complete Block Design</td>
</tr>
<tr>
<td>RDW</td>
<td>Root Dry Weight</td>
</tr>
<tr>
<td>Redox</td>
<td>Reduction-Oxidation Potential</td>
</tr>
<tr>
<td>SAR</td>
<td>Systemic Acquired Resistance</td>
</tr>
<tr>
<td>SE</td>
<td>Standard Error of the Mean</td>
</tr>
<tr>
<td>SDW</td>
<td>Shoot Dry Weight</td>
</tr>
<tr>
<td>TWA</td>
<td>Time Weighted Average</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

Water is a significant limiting natural resource for crop production in nearly all major agricultural regions on Earth (Morison et al., 2008; Pfister et al., 2011; Rosegrant et al., 2009). Although endowed with more freshwater resources than most other places on the planet, Canada is not immune to the crop production challenges associated with inadequate water supplies. The inadequacy of water supplies, in the context of this thesis, refers to both quantity and quality as these two factors combine to determine the 'usefulness' of a water source/supply for irrigation purposes.

Modern agricultural practices are both partially responsible for diminished and degraded water supplies and subject to the problems associated with those deteriorating supplies (Rosegrant et al., 2009; Tilman et al., 2002). Take for instance greenhouse and nursery sectors, which rely heavily on irrigation to produce a marketable commodity. Larger operations in these industries routinely require millions of litres per day (Majsztrik et al., 2011). Facility operators are well aware of the growing cost and dwindling supply of fresh water. Given these trends, coupled with recent legislative developments both domestically and worldwide (Berghaage et al., 1999; Morison et al., 2008; Province of Ontario, 1990, 2002, 2006; Runia, 1994), many operators are now collecting and re-using irrigation solutions. This is an efficient use of available water resources; however, the recaptured water is often degraded and not suitable as an irrigation water source without some level of treatment (Stewart-Wade, 2011).

The passage of water through a nursery or greenhouse system changes the overall composition of the solution (Stewart-Wade, 2011). Water moving through a crop entrains a host of chemical and biological solutes (Stewart-Wade, 2011; Toze, 2006). In the case of fertigation solutions [irrigation solutions containing fertilizer], the nutrient balance will also be modified as the crop and growth substrate retain and leach ions at rates that change over time. The contamination of irrigation runoff waters by chemical and biological vectors necessitates a water treatment process to ensure that the crops are not unduly exposed to potentially detrimental factors (Stewart-Wade, 2011). Ozonation, the dissolution of ozone gas (in the irrigation stream), is one such technology that can be used to control chemical and biological contaminants; however, it is not being used to its full benefit due to an operational convention that directs growers to remove the ozone prior to crop distribution. Justifiable prudence has dictated the removal, as

This thesis follows the style guidelines of the Journal of the American Society for Horticultural Sciences
the phytotoxicity of ozone in the gas phase (as a component of photochemical smog) has been well established (Bell and Treshow, 2002). Having said this, the phytotoxicity of ozone in aqueous solution has not yet been adequately substantiated (in the scientific literature) in the context of irrigation solution management.

Without sufficient evidence to confirm or refute the rationale for removing residual ozone, growers should continue to err on the side of caution and remove ozone prior to crop application. What if, however, the crop was able to safely accommodate some level of residual aqueous ozone? This could lead to improvements in system hygiene [entire distribution system exposed to the treatment] and could have benefits well beyond. The question then is:

*What is the tolerance of plants to ozone in aqueous solution, and is this tolerance threshold high enough to be useful in other aspects of system maintenance and integrated pest management plans?*

Should crop species be sufficiently tolerant to dissolved ozone, it is reasoned that the applied ozone could be sufficient to control pests and/or pathogens demonstrating less tolerance than the crop species in question. One such pest is the common thalloid liverwort *Marchantia polymorpha* L., a primitive plant that is a common weed in temperate greenhouse and nursery operations. Control of *M. polymorpha* is difficult and expensive, but there are grounds to suggest that it may be more sensitive to aqueous ozone than many crop species. Should this prove correct, the routine use of ozonated irrigation water could play a role in keeping *M. polymorpha* in check.

This study was undertaken to address the potential phytotoxicity of ozone in aqueous solution applied directly to greenhouse and nursery crop species. Further, the study sought to evaluate the potential to utilize differences in phytotoxic thresholds between crop species and *M. polymorpha* for the control of said weed species. The principle research questions addressed are:

1) **What is the response of tomato (Solanum lycopersicum L.) grown in hydroponic, mineral wool, drip-irrigation culture to daily applications of aqueous ozone delivered directly to the mineral wool substrate surface?**

2) **What are the responses of select woody nursery perennial species to daily overhead irrigation with aqueous ozone?**
3) Can the plant pest *Marchantia polymorpha* be inhibited with aqueous ozone at exposure frequencies and concentrations that are compatible with crop production?

These studies provided a baseline for the understanding of aqueous ozone as an agricultural water remediation tool with respect to direct crop application, while providing insight into a potential ancillary pest control application.
CHAPTER 2

BACKGROUND AND RATIONALE

In many of the world’s largest greenhouse and nursery production regions, irrigation water supply and management have become significant operational challenges (Johansson et al., 2002; Majsztrik et al., 2011; Rosegrant et al., 2009; Van Os, 1999; Wallace, 2000). Increasingly restricted and degraded water supplies, coupled with the perennial threat of existing and emerging disease and pest vectors, presents significant obstacles to nursery and greenhouse production (Hong and Moorman, 2005; Johansson et al., 2002; Majsztrik et al., 2011). These production barriers are exacerbated by consumer and legislative demands that are limiting the ability of production managers to deal with the issues at hand (Province of Ontario, 1990, 2002, 2006; Tose, 2006; Yiridoe et al., 2005).

Consumers have become more conscious of chemical and resource use, while evolving government regulations will significantly restrict or alter traditional agricultural water use and pest control practices (Johansson et al., 2002; Uri, 1998; Rosegrant et al., 2009; Toze, 2006; Yiridoe et al., 2005). Include the consequences of global climate change and its potential influence on water demand and availability, as well as the distribution and emergence of new pests and pathogens (Boland et al., 2004; Johansson et al., 2002), it becomes clear that nursery and greenhouse managers require new technologies and management strategies to meet these resource and pest challenges as well as the environmental, social and legislative shifts facing the industry (Daughtrey and Benson, 2005; Hong and Moorman, 2005).

It is no longer feasible to discard irrigation solutions after a single pass through the production system, be it on social, economic, environmental or legislative grounds. Many greenhouse operators now collect irrigation run-off for reapplication to the crop (Richard et al., 2006; Stewart-Wade, 2011). Although this is an efficient use of available resources it presents problems in terms of deteriorating solution quality, including nutrient imbalances and an increased risk of pathogen proliferation (Fig. 2.1) (Daughtrey and Benson, 2005; Hong and Moorman, 2005; Johansson et al., 2002; Stewart-Wade, 2011; Van Os, 1999). The risk of pathogen proliferation associated with irrigation water recycling is currently the biggest impediment to adoption of these recycling systems (Richard et al., 2006). Development of effective water and pest management strategies that address these issues while delivering savings in an environmentally benign fashion are an important component of future greenhouse and nursery management practices.
Fig. 2.1: Risk of disease propagation in nursery and greenhouse irrigation systems that collect and reapply irrigation solutions without preventative treatment measures in place. Initially clear water is applied to a crop in which an infection has established in a small number of plants (red pots in top image). The infected plant sheds pathogen propagules that are carried away by the irrigation run-off. The collected run-off water, now carrying a pathogen load, is reapplied to the crop. Viable infectious propagules are distributed to previously uninfected plants; the disease spreads with each successive watering event. This continued re-inoculation could eventually lead to complete crop failure.
IRRIGATION SOURCE WATER REMEDIATION

Regardless of the source of the irrigation water, be it captured run-off or water new\(^2\) to the system; conditioning is required (Ehret et al., 2001; Stewart-Wade, 2011). This can be as simple as a coarse-filtering step for large particulate removal to technologically sophisticated water treatment systems capable of removing all but the most recalcitrant of chemical and biological contaminants. The scope of the systems employed will depend on source water quality, overall goals of the system, and economic feasibility.

There are numerous options available to greenhouse and nursery operators for irrigation water treatment, many of which are detailed in Table 2.1, and further discussed in reviews by Ehret et al. (2001), Stewart-Wade (2011) and Runia (1995). A common misconception is the assumption that a single technology will address all the water quality issues associated with a particular water source. The misconception is stretched further when multiple water sources are used in a single production system (e.g. re-cycled water and rain water). Each technology has its strengths and drawbacks (Ehret et al., 2001; Stewart-Wade, 2011) and will be more or less appropriate for any given source water or production system.

Ozone is attractive as an irrigation water remediation technology for numerous reasons, including its broad-spectrum efficacy (biological and chemical contaminants), and the lack of residues on the crop. There are, however, concerns regarding the potential for crop damage due to ozone off-gassing from aqueous solution.

OZONE IN GREENHOUSE AND NURSERY PRODUCTION – AN OVERVIEW

Ozone has been used in water treatment for over 100 years with the first large-scale applications being in municipal water treatment (Table 2.1, 2.2). Since those early days the range of applications for ozone (aqueous and gas) has expanded greatly, with the most significant advances being made in the last 20-30 years. In an agricultural context, ozone-based technologies and applications continue to experience slow but steady growth (Sopher et al., 2002). Current and emerging applications include the extension of post-harvest shelf life in commodities as diverse as wheat (Wu et al., 2006) and cut roses (Robinson et al., 2009), soil fumigation (Larson, 2002), and the remediation of irrigation water in greenhouse and nursery operations (Bourbos and Barbopoulou, 2005; Ehret et al., 2001; McDonald, 2007; Stewart-Wade, 2011).

---

\(^2\) New does not imply that the water is suitable for irrigation purposes.
Table 2.1: Summary of established irrigation and nutrient solution treatment systems currently used in the greenhouse and nursery industries (Adapted from Stewart-Wade, 2011).

<table>
<thead>
<tr>
<th>TREATMENT TECHNOLOGY</th>
<th>ADVANTAGES</th>
<th>DISADVANTAGES</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PHYSICAL SYSTEMS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sedimentation (including electro-coagulation)</td>
<td>Simple Safe(^a) Accommodates variable water conditions</td>
<td>Sludge removal and disposal () Removes beneficial microbes</td>
</tr>
<tr>
<td>Slow Filtration - Sand</td>
<td>Simple Safe Low tech(^b) Low energy Accommodates variable water conditions Retains natural micro-flora No by-products/residuals No pre-filter required</td>
<td>High setup costs Too slow for high volume Clog quickly Backwashing required High maintenance requirements (Legionella) in microbial community Efficacy breakdowns Mass intensive</td>
</tr>
<tr>
<td>Slow Filtration - Mineral wool</td>
<td>Simple Safe Low tech Low energy Accommodates variable water conditions Light (cf. sand) More efficacious with certain pathogens</td>
<td>High setup costs More complex than sand Too slow for high volume throughput Efficacy breakdown (Legionella) bacteria part of microflora</td>
</tr>
<tr>
<td>Slow Filtration - Pumice</td>
<td>Simple Safe Low tech Low energy Accommodates variable water conditions Light (cf. sand) High filtration capacity Less clogging/lower maintenance More efficacious with certain pathogens</td>
<td>High setup costs More complex than sand Too slow for high volume throughput Occasional efficacy breakdown (Legionella) in microbial community</td>
</tr>
<tr>
<td>Membrane Filtration</td>
<td>Highly effective Safe Not pH dependent</td>
<td>Not typically considered practical (high costs, rapid fouling, high labour requirements)</td>
</tr>
<tr>
<td>Heat</td>
<td>Simple Safe Low tech No harmful by-products Well establish (Europe) Intuitive</td>
<td>Expensive (energy costs) Need to cool water afterwards Need to acidify water (scale prevention) Kills beneficial microbes Corrosive</td>
</tr>
<tr>
<td>Ultraviolet Radiation</td>
<td>Safe Non-corrosive Not pH dependent</td>
<td>Highly affected by solids Pre-filter required Lamp replacement costs Kills beneficial microbes Breaks down iron chelates</td>
</tr>
</tbody>
</table>

\(^a\) -- For the purposes of this discussion safe refers to the lack of chemical use.
\(^b\) -- Systems can be built/installed by laypersons – requires no specialized skills.
<table>
<thead>
<tr>
<th><strong>TREATMENT TECHNOLOGY</strong></th>
<th><strong>ADVANTAGES</strong></th>
<th><strong>DISADVANTAGES</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CHEMICAL SYSTEMS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorine</td>
<td>Highly effective</td>
<td>Affected by solids</td>
</tr>
<tr>
<td></td>
<td>Residual for continued disinfection</td>
<td>Affected by pH</td>
</tr>
<tr>
<td></td>
<td>Effective on biofilms and algae</td>
<td>Known harmful DBPs</td>
</tr>
<tr>
<td></td>
<td>Well established</td>
<td>Phytotoxic at higher levels</td>
</tr>
<tr>
<td></td>
<td>Easily automated</td>
<td>Corrosive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Handling and storage difficult</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chlorine gas very hazardous</td>
</tr>
<tr>
<td>Chlorine Dioxide</td>
<td>Broader pH range (cf. chlorine)</td>
<td>Human and environmental hazards</td>
</tr>
<tr>
<td></td>
<td>Biocidal action not affected by nitrogenous compounds (cf. chlorine)</td>
<td>Lack of phytotoxicity data</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lack of efficacy data</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Must be produced and used on site</td>
</tr>
<tr>
<td>Bromine</td>
<td>Effective even at higher pH</td>
<td>Affected by variable water conditions</td>
</tr>
<tr>
<td></td>
<td>Biocidal action not affected by nitrogenous compounds</td>
<td>Potential human and environmental hazards</td>
</tr>
<tr>
<td></td>
<td>Low/no phytotoxicity</td>
<td>Phytotoxic</td>
</tr>
<tr>
<td></td>
<td>Less persistent DBP (cf. chlorine)</td>
<td>Corrosive</td>
</tr>
<tr>
<td></td>
<td>Easily automated</td>
<td>Handling and storage difficult</td>
</tr>
<tr>
<td>Hydrogen Peroxide</td>
<td>Simple</td>
<td>Affected by variable water conditions</td>
</tr>
<tr>
<td></td>
<td>Well established</td>
<td>Potential human and environmental hazards</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phytotoxic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Corrosive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Handling and storage difficult</td>
</tr>
<tr>
<td>Ozone</td>
<td>Low environmental impact</td>
<td>Low residual disinfection capacity</td>
</tr>
<tr>
<td></td>
<td>No residual on crop/produce</td>
<td>Potential for phytotoxicity (off gas)</td>
</tr>
<tr>
<td></td>
<td>Supplies oxygen to root zone</td>
<td>Potential health hazard (gas leaks)</td>
</tr>
<tr>
<td></td>
<td>Improves production (?)</td>
<td>Corrosive (hardware compatibility)</td>
</tr>
<tr>
<td></td>
<td>Controls chemical contaminants</td>
<td>Potential influence on Fe and Mn</td>
</tr>
<tr>
<td>Fungicides</td>
<td>Well studied</td>
<td>Potential phytotoxicity</td>
</tr>
<tr>
<td></td>
<td>Effective</td>
<td>Human and environmental hazards</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Resistance development</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Can be expensive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Applicator licensing</td>
</tr>
<tr>
<td><strong>BIOLOGICAL SYSTEMS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biological Control Agents</td>
<td>Pathogen specific</td>
<td>Specificity may limit applicability</td>
</tr>
<tr>
<td></td>
<td>Improved plant growth (?)</td>
<td>Lack of efficacy data (water based)</td>
</tr>
<tr>
<td></td>
<td>Do not interfere with other treatments</td>
<td>Stability/reliability?</td>
</tr>
<tr>
<td>Biofilters</td>
<td>Safe</td>
<td>Removes excess nutrients</td>
</tr>
<tr>
<td></td>
<td>Retains natural microflora</td>
<td>Little data available for nursery/greenhouse systems</td>
</tr>
<tr>
<td></td>
<td>Faster flow rates (cf. sand)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Removes excess nutrients</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Minimal/no phytotoxicity</td>
<td></td>
</tr>
<tr>
<td>Constructed Wetlands</td>
<td>Safe</td>
<td>Potential for salinity build-up</td>
</tr>
<tr>
<td></td>
<td>Removes pesticides and excess nutrients</td>
<td>Nutrient removal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Potentially large area requirements</td>
</tr>
</tbody>
</table>

---

- Depending on the system and nutrients in question the removal can be a benefit or detriment.
Table 2.2: Ozone physical, chemical and regulatory data

<table>
<thead>
<tr>
<th>PROPERTY</th>
<th>VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CHEMICAL/PHYSICAL</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Molecular Formula</td>
<td>O&lt;sub&gt;3&lt;/sub&gt;</td>
</tr>
<tr>
<td>Molar Mass</td>
<td>47.9982 g·mol&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Density</td>
<td>2.144 g·L&lt;sup&gt;-1&lt;/sup&gt; at 0ºC</td>
</tr>
<tr>
<td>Melting Point</td>
<td>-192.5ºC</td>
</tr>
<tr>
<td>Boiling Point</td>
<td>-111.9ºC</td>
</tr>
<tr>
<td>Solubility in H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>0.64 (β; v/v); 0.104g/100ml at 0ºC</td>
</tr>
<tr>
<td>Specific Gravity</td>
<td>1.66</td>
</tr>
<tr>
<td>Critical Pressure</td>
<td>5 460 kPa (abs)</td>
</tr>
<tr>
<td>Critical Temperature</td>
<td>-12.1ºC</td>
</tr>
<tr>
<td>Approximate Half Life</td>
<td></td>
</tr>
<tr>
<td>Air&lt;sup&gt;b&lt;/sup&gt;</td>
<td>~ 4-12 hrs</td>
</tr>
<tr>
<td>Water&lt;sup&gt;c,e&lt;/sup&gt;</td>
<td>&lt;1 to &lt;20 minutes</td>
</tr>
<tr>
<td><strong>REGULATORY/SAFETY</strong>&lt;sup&gt;a,d,e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Detectable odour</td>
<td>0.005-0.04 ppm,</td>
</tr>
<tr>
<td>Health and Safety Limits (Canada)</td>
<td>0.1 ppm, TWA (8hr, 5 days/wk) – light work</td>
</tr>
<tr>
<td></td>
<td>0.08 ppm&lt;sub&gt;v&lt;/sub&gt;, TWA (8hr, 5 days/wk) – moderate work</td>
</tr>
<tr>
<td></td>
<td>0.05 ppm&lt;sub&gt;v&lt;/sub&gt;, TWA (8hr, 5 days/wk) – heavy work</td>
</tr>
<tr>
<td></td>
<td>Short Term Limit: 0.3 ppm&lt;sub&gt;v&lt;/sub&gt;, over 15 minutes, not to be repeated more than 2x in an 8hr period</td>
</tr>
<tr>
<td>Chest pain, dry cough, lung irritation, severe fatigue</td>
<td>0.6-1.0 ppm&lt;sub&gt;v&lt;/sub&gt;, over 1-2 hrs</td>
</tr>
<tr>
<td>Immediately Dangerous to Life and Health (IDLH)</td>
<td>5 ppm&lt;sub&gt;v&lt;/sub&gt;</td>
</tr>
<tr>
<td>Expected to be fatal</td>
<td>50 ppm&lt;sub&gt;v&lt;/sub&gt; (30 minutes)</td>
</tr>
</tbody>
</table>

a – Source: Rakness, 2005.
b – Highly variable depending on the purity and temperature of the air
c – Highly variable depending on the purity, temperature and pH of the water.
d – Source: Canadian Centre for Occupational Health and Safety
Amongst the aforementioned applications, the treatment of irrigation water is perhaps the most transformative when recognizing that water is a major limiting resource (quality and/or quantity) in protected crop production (Majsztrik et al., 2011). Accelerating population growth and shifting climactic regimes will intensify the pressure on global fresh water supplies, in many cases to the point of acute scarcity (Johansson et al., 2002; Seckler et al., 2001). Many cropping systems will not be sustainable in this emerging water reality without comprehensive changes to current irrigation practices, including but not limited to a shift to capture and re-use systems (Majsztrik et al., 2011).

Ozonation is one of many technologies available for treating irrigation solutions (Stewart-Wade, 2011; Ehret, 2001). As detailed in Table 2.1, ozone is attractive as an irrigation water treatment technology due to its strong oxidation potential, high microbiocide activity, and relative ease of use (cf. compounds with higher reductive potentials; Table 2.3). Further, ozone leaves no residual on the crop, as any ozone not consumed through microbial inactivation or reaction with chemical contaminants spontaneously reverts to diatomic oxygen through a complex decomposition mechanism (Fig. 2.2; Appendix 1). This decomposition further enhances the effectiveness of the treatment through the production of hydroxyl radicals (Table 2.3; Fig. 2.2; Appendix 1), which are capable of reacting with the more recalcitrant contaminants. The lack of a chemical residue makes ozonation a more socially palatable disinfection option.

It should also be noted that the process of generating, dissolving, and decomposing ozone in solution has the ancillary benefit of super-saturating the solution with dissolved diatomic oxygen (O$_2$). Many growers actively aerate their irrigation solutions to prevent disease and to ensure adequate root zone oxygenation. Systems employing ozonation would, therefore, reduce or eliminate the need for supplemental aeration infrastructure. Further, some studies suggest that moderately super-saturated solutions can improve production, albeit only slightly (Zheng et al., 2007).

Another attractive feature of ozone as an irrigation solution disinfection technology is pathogen resistance development is improbable. Traditional pesticides tend to act on a single metabolic pathway to disrupt a pathogen. This generates a significant selective pressure on the pathogen, ultimately leading to the evolution of resistance. Conversely, ozone reacts with a wide range of cellular components (predominantly components of the cell membrane), leaving a pathogen broadly exposed to cellular disruption (Cho et al., 2010; Zhang et al., 2011).
Table 2.3: Redox potentials of some key oxidative species related to water treatment.

<table>
<thead>
<tr>
<th>CHEMICAL SPECIES</th>
<th>HALF CELL REACTION (ACID SOLUTIONS)</th>
<th>$E^0$ ( VOLTS)$^a$</th>
<th>RELATIVE POTENTIAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorine</td>
<td>$F_2(g) + 2H^+ + 2e^- \rightleftharpoons 2HF$</td>
<td>3.053</td>
<td>1.46</td>
</tr>
<tr>
<td></td>
<td>$F_2(g) + 2e^- \rightleftharpoons 2F^-$</td>
<td>2.87</td>
<td>1.38</td>
</tr>
<tr>
<td>Hydroxyl Radical</td>
<td>$HO^- + H^+ + e^- \rightleftharpoons H_2O$</td>
<td>2.76</td>
<td>1.33</td>
</tr>
<tr>
<td>Atomic Oxygen</td>
<td>$O(g) + 2H^+ + 2e^- \rightleftharpoons H_2O$</td>
<td>2.43</td>
<td>1.17</td>
</tr>
<tr>
<td>Ozone</td>
<td>$O_3(g) + 2H^+ + 2e^- \rightleftharpoons O_{2(g)} + H_2O$</td>
<td>2.076</td>
<td>1.00</td>
</tr>
<tr>
<td>Hydrogen Peroxide</td>
<td>$H_2O_2 + 2H^+ + 2e^- \rightleftharpoons 2H_2O$</td>
<td>1.763</td>
<td>0.85</td>
</tr>
<tr>
<td>Permanganate</td>
<td>$MnO_4^- + 4H^+ + 3e^- \rightleftharpoons MnO_{2(s)} + 2H_2O$</td>
<td>1.695</td>
<td>0.81</td>
</tr>
<tr>
<td>Hypochlorous Acid</td>
<td>$HClO + H^+ + e^- \rightleftharpoons \frac{1}{2}Cl_2(g) + H_2O$</td>
<td>1.630</td>
<td>0.79</td>
</tr>
<tr>
<td>Hypobromous Acid</td>
<td>$HBrO + H^+ + e^- \rightleftharpoons \frac{1}{2} Br_2 + H_2O$</td>
<td>1.604</td>
<td>0.77</td>
</tr>
<tr>
<td>Hydroperoxyl Radical</td>
<td>$HO_2^- + H^+ + e^- \rightleftharpoons H_2O_2$</td>
<td>1.44</td>
<td>0.69</td>
</tr>
<tr>
<td>Chlorine</td>
<td>$Cl_{2(g)} + 2e^- \rightleftharpoons 2Cl^-$</td>
<td>1.358</td>
<td>0.65</td>
</tr>
<tr>
<td>Molecular Oxygen</td>
<td>$O_2 + 4H^+ + 4e^- \rightleftharpoons 2H_2O$</td>
<td>1.229</td>
<td>0.59</td>
</tr>
<tr>
<td>Iodate</td>
<td>$IO_3^- + 6H^+ + 5e^- \rightleftharpoons \frac{1}{2} I_{2(s)} + 3H_2O$</td>
<td>1.195</td>
<td>0.58</td>
</tr>
<tr>
<td>Superoxide</td>
<td>$O_2^- + H_2O + e^- \rightleftharpoons HO_2^- + OH^-$</td>
<td>0.20</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Fig. 2.2: Generalized ozone decomposition pathway in aqueous solution. The cycle is initiated by the reaction of ozone ($O_3$) with a hydroxide ion ($OH^-$) or some other promoter (e.g. UV, $H_2O_2$). Once started the decomposition is rapid and self-sustaining (Propagation) provided there are no compounds that will react with the hydroxyl radical ($OH^-$) without generating another radical species (Termination). Adapted from Glaze (1986) and Beltrán (2004). See also Appendix 1.
Although ozone as an irrigation solution remediation technology has many attractive features, there are also drawbacks and significant knowledge gaps preventing operators from realizing its potential. Additional research into ozonation as an irrigation solution remediation technology is required to allow operators to better assess the promise of this technology in comparison to the other technologies listed in Table 2.1 (Stewart-Wade, 2011). Data pertaining to impacts on nutrient ions, and the phytotoxicity of ozone in the aqueous phase is grossly inadequate for growers to make an informed decision regarding the maintenance of residual ozone during distribution of irrigation solutions to the crop. In light of this paucity of data, growers wisely err on the side of caution and remove residual ozone prior to crop application. Although sagacious given the available data, the removal of ozone greatly limits the capacity of the treatment to control contamination throughout the system. Further, it eliminates any potential to realize ancillary benefits downstream of the primary treatment site.

**OZONE SOLUBILITY – INFLUENCING FACTORS**

In order to use ozone as an irrigation solution remediation tool, the gas must first be dissolved in the solution. Like most gases, the factors regulating the solubility of ozone in water are complex and multifaceted. The most significant of these thermodynamic and physical chemistry factors are presented here. For a detailed treatise of these and other regulating factors, the reader is directed to Appendix 1.

**The Influence of Temperature on Ozone Solubility**

Solution temperature is typically the most influential factor on ozone solubility (Fig. 2.3). The basic thermodynamics governing gas solubility are well known and discussed in detail in Appendix 1. Briefly, the solubility of ozone is inversely proportional to the solution temperature (Fig. 2.3). Although the dissolution of ozone (most gasses) is enthalpically favourable (exothermic), the relative contribution of the enthalpic term, in the calculation of the thermodynamic equilibrium constant (K), decreases as temperature increases (Bunce, 1993). Eventually, as solution temperature increases, the entropic contribution to K exceeds the enthalpic term and the dissolution of gas will no longer be thermodynamically favourable (Bunce, 1993) (see Appendix 1).
Fig. 2.3: The solubility of ozone as influenced by solution temperature and gas phase ozone concentration at 101.3 kPa pressure. Data for graph extracted from Harrison (2004).
Henry’s Law and Ozone Solubility – Gas Phase Concentration and Pressure

Henry’s Law\(^3\) describes the equilibrium distribution, for some molecule or compound, between the bulk liquid and bulk gas phases (Bunce, 1993; Sotelo et al., 1989a). For ozone, this equilibrium is represented by Eq. [2.1].

\[
\text{O}_3(\text{g}) \rightleftharpoons \text{O}_3(\text{aq}) \quad \text{Eq. [2.1]}
\]

Essentially, Henry’s Laws states that the concentration of the gas in solution is proportional to the partial pressure (e.g. concentration) of the gas in contact with the solution (Fig. 2.3) and is described (for ozone; Sotelo et al., 1989a) by Eq. [2.2].

\[
[\text{O}_3(\text{aq})] = K_H \cdot p(\text{O}_3(\text{g})) \quad \text{Eq.[2.2]}
\]

where;  
- \([\text{O}_3(\text{aq})]\) is the ozone gas concentration in water with units of mol⋅L\(^{-1}\) (or equivalent)  
- \(K_H\) is the Henry’s Law/Equilibrium constant with units of mol⋅L\(^{-1}\)⋅atm\(^{-1}\) (or equivalent)  
- \(p(\text{O}_3(\text{g}))\) is the partial pressure of ozone gas with units of atmosphere (or equivalent)

The critical implication of this relationship, in terms of ozone water treatment, is the fact that the higher the partial pressure of ozone gas, the greater the aqueous concentration. Depending on the application, the implications of Henry’s Law will dictate the type and size of ozone generator required to meet the ozone demands of the source water.

Ozone is a highly reactive gas; further, in aqueous solution the half-life of ozone is significantly lower than in the gas phase (Table 2.2; Fig. 2.2). As a result, the equilibrium described in equation 2.1 is rarely achieved. This also influences ozonation system design and generator selection.

Solution pH

As a general rule of thumb, ozone is more stable in acidic solutions than basic (Beltrán, 2004). The decomposition of ozone in aqueous solution is initiated by the hydroxide ion (OH\(^-\)) (Fig. 2.2; Appendix 1), and as such, the ozone decomposition rate increases as pH rises ([OH\(^-\)] increases) (Beltrán, 2004). This increased rate of ozone decomposition further disrupts the equilibrium in equation 2.1.

\(^3\) The relationship was formulated by William Henry in 1803.
The accelerated decomposition of ozone at elevated pH levels is one means to achieve advanced oxidation. Advanced oxidation processes (AOP) are actually a combination of processes specifically designed to generate hydroxyl radicals (OH *) for the oxidation of target contaminants or compounds (Beltrán, 2004; Gottschalk et al., 2000). Other examples of ozone-related AOPs are O3/UV, O3/hydrogen peroxide (H2O2), and O3/UV/titanium dioxide (TiO2) (Beltrán, 2004; Gottschalk et al., 2000).

Salt Content

It is well known that the presence of inorganic ions (salts) affect the solubility of gases in solution (Rischbieter et al., 2000; Ruckenstein and Shulgin, 2002; Sotelo et al., 1989b). In the case of aqueous ozone systems, the effect is typically a lowering of solubility (salting-out effect). The influence of ionic components on the solubility of a gas are described by the Sechenov Equation [Eq. 2.3], which relates the influence of overall ionic strength, and the make up of the ion and gas mix, to an 'apparent' Henry's Law constant (KH-app) (Ruckenstein and Shulgin, 2002).

\[
KH_{-app} = KH_{-sfw} \cdot 10^{KsC_s}
\]  
Eq. [2.3]

where: 
- \( KH_{-app} \) is the apparent Henry's constant
- \( KH_{-sfw} \) is the Henry's constant in salt-free water
- \( K_s \) is the Sechenov constant specific to the gas and salt
- \( C_s \) is the concentration of the salt

In dilute solutions (see Appendix 1), the influence of ionic constituents on \( KH \) is minimal and largely ignored in all but the most precise applications. Although ionically complex, a typical irrigation solution is relatively dilute (Appendix 1), so as to maintain an osmotically favourable environment for water uptake by the roots. In most production systems, the influence of salting-out effects are ignored or only given a cursory consideration.

Plant Nutrients and Ozonation

Ozone is a strong oxidizer and, as such, it may interact with fertilizer ions when used as an irrigation water treatment, affecting productivity indirectly through modified nutritional regimes. Previous studies have demonstrated that the major plant nutrient ions (e.g. macronutrients) are not affected by ozonation (Ehret et al., 2001; Graham, 2001; McDonald, 2007; Ohashi-Kaneko et al., 2009; Runia, 1994).
Although the macronutrient ions are stable under solution ozonation, the same cannot be said for some of the transition metal micronutrient ions, specifically iron (Fe) and manganese (Mn) (Ohashi-Kaneko et al., 2009; Vanachter et al., 1987). The significance of this incompatibility between these micronutrients and ozone is highlighted by the fact that ozone is commonly used to specifically remove these elements from drinking water (Beltran, 2004; Gottschalk et al., 2000; Rakness, 2005).

Iron

Iron is soluble in water, and most readily available to plants, as ferrous iron (Fe$^{2+}$) (Christ, 1974; Harrison, 2004). In its elemental form, Fe$^{2+}$ is easily oxidized by ozone to form ferric iron (Fe$^{3+}$) which slowly hydrolyzes to form insoluble ferric hydroxide (Fe(OH)$_3$) (Harrison, 2004; Rakness, 2005). The removal of Fe from solution, as a result of ozonation, is a significant concern, particularly in hydroponic systems that use the irrigation solution to deliver nutrients to the crop (fertigation). These losses can, however, be largely controlled with the use of ozone-stable chelates, such as ethylenediaminetetraacetic acid (EDTA) and diethylene triamine pentaacetic acid (DTPA) (Vanachter et al., 1987). Further, the rate of Fe$^{2+}$ oxidation by ozone is reduced at pH values less than about 7.5 (Harrison, 2004). Nutrient solutions are usually maintained at pH values below 7, which further controls the potential loss of Fe from solution under ozonation.

Manganese

Manganese, as with iron, is soluble and available to plants as the divalent cation Mn$^{2+}$ (El-Jaoual and Cox, 1998; Harrison, 2004). Manganese is also available to plants, to a lesser extent, as permanganate (MnO$_4^-$) (University of Florida, 2012). Ozone rapidly oxidizes Mn$^{2+}$ to the tetravalent form (Mn$^{4+}$), which in turn rapidly hydrolyzes to insoluble manganese oxydihydroxide (MnO(OH)$_2$) and manganese dioxide (MnO$_2$) (Gottschalk et al., 2000; Harrison, 2004; Rakness, 2005). If excessive ozone or prolonged ozonation occurs, both Mn$^{2+}$ and Mn$^{4+}$ can be oxidized to permanganate (MnO$_4^-$) (Gottschalk et al., 2000; Harrison, 2004; Rakness, 2005). Permanganate can readily be reduced back to Mn$^{2+}$ with granulated carbon filters, thereby restoring the most available form of the element (Harrison, 2004). The potential nutritional issues with the oxidation of Mn$^{2+}$ can, as with Fe, be largely resolved using ozone-stable chelates (e.g. EDTA)(Vanachter et al., 1987).

Gaseous Ozone Phytotoxicity

Irrigation solutions containing aqueous ozone are a potential source of gaseous ozone in greenhouse and nursery production environments. Ozone can enter the atmosphere through off-gassing (ozone-
containing solutions that are exposed to the bulk atmosphere via spaying etc.), or the venting of excess ozone during the dissolution process without proper handling systems in place (Gottschalk, 2000; Rakness, 2005) (see also Appendix 1). Gaseous ozone could also enter the atmosphere through cracks or leaks in the ozone generator or the plumbing connecting it to the mass transfer system (Harrison, 2004; Rakness, 2005).

Although any direct symptoms of aqueous ozone phytotoxicity have not been characterized in the scientific literature (McDonald, 2007; Sloan and Engelke, 2005), the symptoms of gaseous ozone phytotoxicity are well recognized (Bell and Treshow, 2002; Krupa et al., 2001; Manning and Godzik, 2004). These can include such general symptoms as yellowing and necrosis, which are common to many biotic and abiotic stressors, to more specific symptoms, such as flecking, pigmentation, and stippling (Krupa et al., 2001; Manning and Godzik, 2004)(Table 2.4).

The types of symptoms displayed are also dependent upon the type of exposure. Generally there are two main exposure classifications: 1) acute exposures consisting of relatively high ozone concentrations (e.g. >80 nL\cdot L^{-1}) over a relatively short period of time (e.g. a few hours to days); and 2) chronic exposures consisting of relatively low ozone concentrations (e.g. <40 nL\cdot L^{-1}) over the entire life of the plant (may be punctuated with acute exposures as well) (Krupa et al., 2001). General symptoms, for both regimes, are summarized in Table 2.4.

**ENTRY AND SIGNALLING**

Although there is little scientific evidence to suggest that significant amounts of ozone will come out of solution and into the plant canopy (McDonald, 2007), the basic thermodynamics and physical chemistry of the system (Appendix 1) suggest that it should still be treated as a concern.

Any ozone coming out of an applied aqueous ozone solution must enter the plant before any phytotoxicity can be realized. Uptake by the plant is only one of several sinks including cuticle and soil deposition as well as quenching by volatile compounds emitted by plants (Bell and Treshow, 2002; Holopainen, 2004). This being said, ozone is biologically active at low concentrations (i.e. < 80 ug\cdot L^{-1}) when the exposures are on the order of hours to days (Krupa et al., 2001).
Table 2.4: Visual symptoms of phytotoxicity resulting from gaseous ozone exposure. (Adapted from Krupa et al., 2001; Manning and Godzik, 2004).

<table>
<thead>
<tr>
<th>EXPOSURE REGIME</th>
<th>ACUTE</th>
<th>CHRONIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleaching</td>
<td><img src="bleaching.png" alt="Image" /></td>
<td>Pigmentation (reddening; bronzing; purple; accumulation of pigment compounds/ phenolics)</td>
</tr>
<tr>
<td>(upper, lower or bifacial; small, unpigmented a/o necrotic spots) <em>(Cucurbita spp.)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flecking</td>
<td><img src="flecking.png" alt="Image" /></td>
<td>Chlorosis (from non-green pigmentation or directly from chlorophyll breakdown)</td>
</tr>
<tr>
<td>(small necrotic irregular areas; metallic or brown, becoming tan, grey or white)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stippling</td>
<td><img src="stippling.png" alt="Image" /></td>
<td>Premature senescence (early loss of leaves, flowers or fruit)</td>
</tr>
<tr>
<td>(small circular spots; white, black, red, red-purple)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Banding</td>
<td><img src="banding.png" alt="Image" /></td>
<td>Mottling (diffuse chlorotic regions interspersed with green tissue) <em>(Pinus spp.)</em></td>
</tr>
<tr>
<td>(conifers; clear or chlorotic bands on semi-mature needles) <em>(Pinus spp.)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Necrosis</td>
<td><img src="necrosis.png" alt="Image" /></td>
<td></td>
</tr>
<tr>
<td>(spots of dead tissue that progress and merge into larger and larger areas); <em>(Citrullus lanatus)</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Gas exchange in plants, including the entry of ozone, is regulated by the stomata. Any biotic or abiotic factors influencing the stomata will also influence ozone uptake. As an example, under water stress conditions, which tend to coincide with peak tropospheric ozone levels (smog), the stomata close to conserve water, which also limits ozone entry.

When stomata are open and ozone is present, the gas enters through the stomatal aperture and into the sub-stomatal cavity (Krupa and Manning, 1988) (Fig. 2.4). Once in the sub-stomatal cavity ozone must enter into the aqueous matrix of the apoplast (Fig. 2.4) before it elicits a plant response. As previously discussed, ozone is unstable in aqueous solution, quickly breaking down to form several reactive oxygen species (ROS) (Fig. 2.2; Appendix 1). It is generally though that it is these ROS, rather than ozone itself, that stimulate a response by the plant (Evans et al., 2005), although ozone may react directly with cell membrane components (Kurpa and Manning, 1988). Given a sufficient exposure, the symptoms outlined in Table 2.4 may be demonstrated.

OZONE SAFETY CONCERNS – HUMAN HEALTH AND WORKER EXPOSURE

Ozone, as with any oxidizing gas, is toxic above certain concentration thresholds (Harrison, 2004). Although a properly designed ozonation system will pose little risk to worker health and safety, failures do occur and the hazards must be appreciated. The most probable source of dangerously high ozone exposures are cracks or leaks in the lines leading from the ozone generator to the mass transfer system, or from the mass transfer system to the off-gas destruction system (Rakness, 2005). A third potential failure point is in the off-gas destruction unit itself. Should this critical safety system fail, ozone gas could rapidly accumulate to dangerous levels. The Canadian Centre for Occupational Health and Safety workplace safety limits are presented in Table 2.2.

Ozone gas has an odour threshold of about 0.02 mg·m$^{-3}$, which is below toxic thresholds for light, medium, and heavy work (Table 2.2), however, desensitization can occur (Gottschalk et al., 2000). Symptoms of ozone exposure vary depending of the level and duration of exposure, with headaches and dryness of the throat, nose and eyes being common symptoms following short exposures (Gottschalk et al., 2000; Government of Canada, 2012). In addition to the symptoms from lower dose exposures, higher concentration exposures can cause delayed lung edema, lassitude, tightness or constriction in the chest, and acid-mouth (Gottschalk et al., 2002). In severe exposures symptoms of dyspnea, coughing, choking sensation, tachycardia, vertigo, lowered blood pressure, severe chest pain, and generalized body pain can be experienced. Prolonged or chronic exposures can suppress lung function and trigger other respiratory disorders such as asthma.
Fig. 2.4: Ozone (O₃) entry and signal stimulation in a leaf. A) ozone enters the leaf through the stomatal aperture and into the sub-stomatal cavity; B) ozone must then dissolve in the aqueous apoplast matrix where it either stimulates a response directly or, more likely, breaks down and produces reactive oxygen species (ROS) in the process. These ROS can act as signals, trigger other signal molecules, or participate in further reactions with compounds such as ascorbate and superoxide dismutase (SOD).
OZONATION SYSTEMS FOR IRRIGATION WATER REMEDIATION – THE BASICS

A basic ozonation system for general water treatment, excluding sewage water, consists of an oxygen source, an ozone generator, a mass transfer (e.g. gas-liquid contactor) system, an ozone waste gas destruction system, pre- and/or post- filters, and all the necessary valves, flow gauges and system monitors (safety and feedback control) required for normal operation (Fig. 2.5). Beyond this, the system can incorporate other treatment technologies (e.g. UV) to target specific treatment objectives, or to take advantage of opportunities for advanced oxidation.

OXYGEN SOURCES AND OZONE GENERATION

Ozone is inherently unstable and must be generated on site and at the time of use. There are numerous methods currently available for generating ozone, although only a few are practical for commercial applications, namely corona discharge, ultraviolet, and more recently electrolytic/electrochemical systems.

Corona Discharge Ozone Generation

Corona discharge ozone generators are currently the standard production method in most medium- to large-scale commercial applications and generate up to 10% wt ozone by weight in a feed gas stream (Harrison, 2004). The basic principle of operation is the establishment of a corona, a zone of ionized gas, between a high voltage (typically 5kV-20kV) and ground electrode, which are separated by a dielectric (e.g. glass or ceramic) and a narrow air gap commonly referred to as the discharge gap (where the corona is established) (Gottschalk et al., 2000; Harrison, 2004; Rakness, 2005). Diatomic oxygen (O$_2$), in air or as a concentrated stream, is passed through the high-energy environment of the discharge gap where some of the diatomic oxygen is split into atomic oxygen (O). A percentage of the atomic oxygen combines with diatomic oxygen in the gas stream forming ozone/triatomic oxygen (O$_3$). Atomic oxygen can also interact with other atomic oxygen and reform O$_2$.

The maintenance of a corona generates significant amounts of heat that must be removed to prevent thermal decomposition of the newly formed ozone (Rakness, 2005). Most corona discharge generators will have large heat sinks in contact with the ground electrode. These heat sinks are typically air cooled, but can also be liquid cooled in high voltage/high frequency systems.
Fig. 2.5: Basic components and flows in a typical aqueous ozone system used for irrigation water treatment. The current practice is to remove ozone prior to distribution to the crop.
The feed gas for corona discharge generators must be free of particulates and other contaminants that could affect the performance of the generator (Harrison, 2004; Rakness, 2005). Further, it is absolutely critical that moisture (i.e. humidity) be removed from the gas stream, as it has two major negative impacts of the generation of ozone. First, moisture in the feed gas will dramatically reduce the amount of ozone generated. As an illustration, the ozone output from a typical corona discharge generator running on ambient air will be half that of a generator running on air with a dew point of -60°C (i.e. the recommended operating dew point) (Harrisonson, 2004). The second concern with high moisture feed gas is the formation of nitric acid, which can severely corrode the electrodes and dielectric materials in the generator. Nitrogen (N₂) in the feed gas is also split into its atomic form. The nitrogen atoms can then react with oxygen to form nitrogen oxides (Harrison, 2004). These nitrogen oxides can dissolve to form nitric acid (Harrison, 2005; Rakness, 2005).

Even in relatively dry ambient air, there is sufficient moisture to cause the problems previously discussed, as such, air preparation is a requirement for any commercial-scale corona discharge generator (Gottschalk et al., 2000; Rakness, 2005). Moisture in ambient air can be removed with sorbents or by heating the air to lower the dew point (Rakness, 2005). Given that feed gas conditioning is required anyway, many operators elect to use gas preparation systems that provide higher O₂ percentages (i.e. >90%) and, as a result, produce greater amounts of ozone per unit of power. The two principle approaches are to; 1) use an oxygen concentrator, or 2) use liquid oxygen (LOX). Oxygen concentrators are the standard for small- to medium-sized commercial applications, whereas LOX is used for large-scale systems (e.g. municipal water treatment plants). An oxygen concentrator works by passing ambient air through molecular sieve columns under pressure (i.e. 207 kPa), removing both nitrogen and water from the gas stream (Harrison, 2004). Most concentrators will have at least two sieve columns that alternate between being pressurized (i.e. concentrating O₂) and depressurized. The depressurization step releases the nitrogen and water, which is vented back to the atmosphere. These systems are referred to as pressure swing adsorption (PSA) generators. Due to the economies of scale required to justify the use of LOX, and the technical skills required to support it, it is typically relegated to operations capable of processing many ML per day, and is not practical for farm applications.

**Ultraviolet Ozone Generators**

Ozone generators utilizing UV radiation operate on the same principle as that acting in the stratosphere to produce the ‘ozone layer’. Short-wave UV radiation (i.e. <200 nm) is absorbed by O₂ and transfers sufficient energy to break the bond between the oxygen atoms (Harrison, 2004). As with the corona discharge generator, the atomic oxygen combines with O₂ to form O₃. Wavelengths greater
than 200 nm are better at breaking ozone down and must be excluded. This is easily accomplished by putting a quartz sleeve between the bulb and the air stream (Harris, 2004).

Ozone production, as a % wt, is lower for UV generators than corona discharge and electrolytic generators. Ozone output from a UV system is typically <1% wt. Although the output is low, the systems are relatively simple and do not have an absolute requirement for feed conditioning (Harrison, 2004). As such, UV systems are useful for small-scale systems.

**Electrochemical Ozone Generators**

The production of ozone via electrolytic processes has been known for as long as ozone has been known (Franco et al., 2008). This being said, it has only been in the last couple of decades that the necessary materials have been developed to allow for reliable, commercial-scale electrochemical generators (Da Silva et al., 2001; Franco et al., 2008). There are two general types of electrochemical ozone generators, which are arbitrarily classified as Type-I and Type-II (Franco et al., 2008). Type-I generators consist of two inert electrodes immersed in an electrolyte solution, the composition of which can be tailored to meet specific needs (Franco et al., 2008). Type-II generators are based on the electrolysis of water and employ a solid polymer electrolyte (SPE) that acts as a proton exchange membrane (PEM) (typically Naphion-117), which is coated on both sides with catalysts that promote the formation of ozone (Han et al., 2006; McDonald, 2007). The anode and cathode for these generators are typically lead dioxide (PbO$_2$) and platinum, respectively (Han et al., 2006). When high purity water is passed over the PbO$_2$ anode at a sufficiently high potential (i.e. >1.51 V) and high current density, ozone will be evolved according to Eq. [2.4].

$$3\text{H}_2\text{O} + 6\text{e}^- \rightarrow \text{O}_3 + 6\text{H}^+ \quad E^\circ = 1.51\text{V} \quad \text{Eq. [2.4]}$$

Protons generated at the anode pass across the PEM and gain electrons at the cathode, forming hydrogen gas [Eq. 2.5].

$$2\text{H}^+ (\text{aq}) + 2\text{e}^- \rightarrow \text{H}_2 (\text{g}) \quad E^\circ = 0.0\text{V} \quad \text{Eq. [2.5]}$$

---

4 Ozone was discovered by Schönbein in 1840 during a water electrolysis experiment
5 PbO$_2$ is an inexpensive and resistant anode material but other materials are gaining favour.
6 This is the standard hydrogen electrode and by convention is assigned a value of zero.
Electrochemical ozone generators are capable of generating very high ozone concentrations (3-47% wt) and do not require feed gas infrastructure, although a source of high purity water is needed as a feedstock for the electrolytic cell(s) (De Silva et al., 2001; Franco et al., 2008). Although the ozone gas concentrations produced are high and can drive greater mass transfer, the technology is not as mature as corona discharge or UV and as such, commercial reliability remains to be established. Further, at higher absolute ozone generation levels, hydrogen gas production/destruction must be addressed, although some researchers have suggested that the hydrogen could act as a fuel source for a fuel cell, thereby off-setting some of the costs of the disinfection system (Han et al., 2006).

**MASS TRANSFER SYSTEM**

*Venturi Injectors*

Venturi injectors are a common method for mixing two fluid streams (liquid/liquid; liquid/gas, gas/gas). These injectors are based on the venturi effect\(^\text{7}\) in which there is a reduction in fluid pressure as a fluid flows past a constriction in a pipe. As water flows through the tapered section of a pipe (i.e. the venturi orifice), a rapid change in velocity occurs. This velocity change creates a vacuum, which draws ozone into the system perpendicular to the direction of liquid flow. The injection rates vary with the pressure differential across the venture orifice (Ozkan et al., 2006) (Fig. 2.5A).

*Static Mixers*

Static mixers are a relatively simple but effective method of fluid mass transfer (Tizaoui and Zhang, 2010). The basic principle of a static mixer (Fig. 2.5B) is to disrupt the fluid flow in a pipe by inserting mixing elements into the flow path (Thakur et al., 2003). The elements redistribute fluid in the directions transverse to the main flow (i.e. in the radial and tangential directions), the efficiency of which is governed by the orientation and number of elements in the static mixer (Thakur et al., 2003). Static mixers are particularly effective in high concentration, low gas flow ozone applications (Tizaoui and Zhang, 2010), such as those employing electrochemical ozone generators.

---

\(^\text{7}\) Discovered by Giovanni Battista Venturi in the 18\(^{\text{th}}\) century
Fig. 2.5: Methods of mass transfer. A) Venturi injectors utilize the vacuum created as a fluid passes through a constriction in the flow path to draw ozone into the process stream. B) Static mixers disrupt the flow direction to achieve vigorous mixing/mass transfer. Images used under the GNU public license. Source: A) http://en.wikipedia.org/wiki/Venturi_effect B) http://en.wikipedia.org/wiki/Static_mixer
**Bubble Columns**

Bubble column ozone contactors are standard mass transfer systems in many municipal water treatment systems, but can be employed in any system that has sufficient room to accommodate the contact column. In a bubble contact column, ozone is injected at the bottom of a water column via a fine bubble diffuser that releases the ozone as small bubbles. The ozone mass transfer occurs via diffusion, which is governed by the size of the bubbles and the height of the water column (Le Sauze et al., 1993; Mitani et al., 2005). The smaller the bubbles, the greater the surface area to volume ratio leading to greater diffusion rates (Le Sauze et al., 1993). The greater the height of the column the longer the bubbles are in contact with the water for diffusion to occur. In more complex bubble column reactors, static mixers and/or venture injectors can be incorporated to reduce the height requirements.

**Off-Gas Destruction System**

Regardless of the generation and mass transfer methods used, there will be ozone that does not transfer into aqueous solution. Further, some of the ozone that goes into solution will also come out of solution. This gas must be properly destroyed to avoid toxicity (human and crop). Fortunately, O₃ is easily converted back to O₂ via any of four methods commonly used in commercial applications.

**Heat**

Ozone exposed to temperatures above 220°C for >5 s will thermally decompose ozone. This method is simple and effective, but does have a large energy requirement (Harrison, 2004).

**UV**

Irradiating ozone with UV light at a wavelength of 254 nm will cause photolysis of the ozone molecule. This method is effective and can be used at low temperatures (Harrison, 2004).

**Granulated Activated Carbon (GAC)**

Passing ozone through a bed of GAC will destroy ozone; however, the process also generates CO₂ and carbon monoxide (CO) and is a consumptive process (i.e. eventually the carbon will all be converted to CO₂/CO). Further, the decomposition process can generate sufficient heat to ignite the carbon resulting in a significant fire hazard. GAC is, however, an effective ozone destruction system for aqueous ozone solutions, where the heat generation is addressed through liquid cooling (Harrison, 2004).
**Manganese Dioxide Catalysis**

Passing ozone through a dry bed of MnO$_2$ catalyst is an efficient method to destroy ozone off-gas. The process is catalytic so the system only needs replacing when fouling or contamination occurs. Although effective, the catalyst must remain dry to retain its ozone destruction capacity. This requires the bed to be heated and/or the incoming gas to be de-watered, both of which add to the system infrastructure requirements (Harrison, 2004).

**MEASURING OZONE**

Developing a high-fidelity ozonation system for the treatment and management of irrigation solutions requires the capacity to know how much ozone is being generated, how much is being dissolved and consumed, and how much is being lost due to incomplete dissolution or as off-gas. There are several methods available for measuring both gaseous and aqueous ozone, and the selection of the technology will depend on both economic and technical considerations of the overall system (Gottschalk et al., 2000; Rakness, 2005). A summary of the available measurement technologies suitable to irrigation water application is presented in Table 2.5.

**UV Absorption**

The recommended method for the measurement of ozone in the gas phase is direct UV absorption (Harrison, 2004). Ozone absorbs UV radiation between approximately 190 – 310 nm with an absorption peak around 254 nm, which is close to the $\lambda = 253.7$ nm mercury resonance line (Daumont et al., 1992; Matsumi and Kawasaki, 2003). Using a mercury lamp, UV is generated at or near the absorption peak of ozone. Introduction of ozone into the sample stream will cause a decrease in the UV intensity at $\lambda = 254$ nm proportional to the ozone concentration in the sample according to the Beer-Lambert law of absorption (Gottschalk et al., 2000).

The basic principle of direct UV absorption can also be used for aqueous phase ozone measurement, although interfering compounds need to be compensated for (Gottschalk et al., 2000; Harrison, 2004). Compensation is achieved by comparing a sample containing both ozone and the interfering compound(s) to a blank sample that does not contain any ozone. The ozone concentration is then based on the difference in absorption between the two samples (Gottschalk et al., 2000; Harrison, 2004).
### Table 2.5: Overview of ozone analytical methods suitable to irrigation water applications

<table>
<thead>
<tr>
<th>DETECTION METHOD</th>
<th>MEASUREMENT RANGE</th>
<th>TYPICAL START-UP COST</th>
<th>MEASUREMENT MODE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrochemical membrane (amperometric)</td>
<td>0-200 mg·L⁻¹ ± 5 ug·L⁻¹</td>
<td>$2,500 – 8,000</td>
<td>aqueous continuous</td>
</tr>
<tr>
<td>Gas stripping with UV detection</td>
<td>0-2 mg·L⁻¹ ± 1%</td>
<td>$5,500 – 8,500</td>
<td>aqueous continuous</td>
</tr>
<tr>
<td>Direct UV detection</td>
<td>0-400 g·m⁻³</td>
<td>$3,000 – 5,000</td>
<td>gas continuous</td>
</tr>
<tr>
<td>Indigo method</td>
<td>0-1.5b mg·L⁻¹</td>
<td>$300 – 3,000</td>
<td>aqueous periodicc</td>
</tr>
</tbody>
</table>

---

a adapted from Bollyky, 2003; Rakness, 2005  
b the range can be increased through dilution or through modifications to the amount of indigo trisulfonate used  
c can be adapted to continuous measurement using flow injection analysis systems (i.e. laboratory applications)

### Indigo Trisulfonate Method

The indigo trisulfonate method, commonly referred to simply as the indigo method, is typically used for grab-sample or periodic analysis of aqueous ozone, but the method can be adapted for continuous monitoring where cost and technical personnel permit (Bollyky, 2003). Ozone concentration is determined by the decolourization of potassium indigo trisulfonate, measured at $\lambda = 600$ nm, upon reaction with ozone. The reaction is fast, has a 1:1 stoichiometric ratio, and is selective for ozone (Bader and Hoigne, 1981; Bollyky, 2003; Gottschalk et al., 2000). The method can be applied in both a volumetric and gravimetric format (Yates and Stenstrom, 2000), making it useful for measurements both in bulk solution and as aqueous ozone is applied to a crop (see Chapter 5).

The indigo method is the basis for the “AccuVac Ampul” system (Hach Company, Loveland, CO.) commonly used in commercial water treatment settings, as well as many research applications. Briefly, all the necessary chemicals for the measurement are pre-packaged under vacuum into a glass vial with an extruded tip. The vial is submerged in the solution being tested and the tip is broken off. The vacuum draws in a sample of solution, dissolving the chemicals and allowing the reactions to proceed. The vial is then used for direct spectrophotometric determination of the ozone concentration (Bollyky, 2003).
Where high concentrations (e.g. >1.5 mg·L\(^{-1}\)) need to be measured and facilities exist, the recipe can be adjusted to increase the amount of indigo trisulfonate available to react with the ozone in the sample. Alternatively, the samples can be diluted with high purity water at the same temperature as the sample, and at a low pH (e.g. < pH 4). These conditions are necessary to ensure that the dilution water is not a major ozone sink, which would influence the reading. The indigo method is the standard used to calibrate other measurement systems such as electrochemical membrane electrodes (Bollyky, 2003).

**Electrochemical Membrane Electrodes**

In terms of aqueous ozone monitoring and control, electrochemical membrane electrodes provide the most flexibility as they provide a continuous, selective, direct measurement of aqueous ozone in an in-line configuration. The basic operational principle behind these sensors is the measurement of a current (amperometry) between two electrodes that is proportional to the concentration of ozone in solution. The electrode itself typically consists of a gold cathode, a silver anode, and an electrolyte solution (e.g. AgBr, K\(_2\)SO\(_4\), or KBr) in which the electrodes are immersed (Gottschalk et al., 2000). The electrode and electrolyte are isolated from the solution being measured by a semipermeable membrane that allows ozone to diffuse into the electrode system (Gottschalk et al., 2000). To prevent depletion of ozone at the membrane surface, the probes are usually inserted into a constant flow cell to ensure continuous replenishment of the dissolved ozone (Rakness, 2005). Ozone is reduced (to O\(_2\)) at the gold cathode [Eq. 2.6]:

\[
O_3 + 2H^+ + 2e^- \rightarrow O_2 + H_2O \quad \text{(cathode reaction)} \quad \text{Eq. [2.6]}
\]

The electrons for the reduction reaction are generated at the silver anode [Eq. 2.7]:

\[
4\text{Ag} \rightarrow 2\text{Ag}^+ + 2e^- \quad \text{(anode reaction)} \quad \text{Eq. [2.7]}
\]

The current generated at the electrons move to the cathode is measured and is proportional to the ozone present in the solution. This system is very selective and provides a rapid and continuous response, and as such it is preferred for larger systems. The sensors are periodically calibrated against the indigo trisulfonate method discussed previously.
Oxidation-Reduction Potential

Oxidation-Reduction Potential (ORP) sensors are mentioned here as they have been used in the past but their non-selectivity has relegated them to use in non-critical applications, such as swimming pool water treatment (Bollyky, 2003). When an oxidant (e.g. ozone, chlorine, bromine) is present in water, there is a small but measureable potential generated, a potential that can be used to characterize the oxidative potential of the solution (Harrison, 2004). Although the method is not specific to ozone, the maintenance of an ORP reading greater than 650-700 mV is considered sufficient to inactivate pathogens (Harrison, 2004).

AQUEOUS OZONE REMOVAL – MISSED OPPORTUNITIES?

Using ozone in a batch treatment format for the remediation of irrigation water can reduce or even eliminate pathogens in that solution (Runia, 1994; Stewart-Wade, 2011). Unfortunately, it has been estimated that only 2-19% of the total microbes found in a hydroponic system are actually in the bulk water (Garland, 1994). The majority of the microbes (e.g. 70 – 90%) are resident in the root zone, with the remaining 1-14% being found in biofilms lining the surfaces of system hardware (e.g. tubing, valves, etc.) (Garland, 1994; Strayer, 1994). If ozone could be left in the solution and applied to the crop, the potential for controlling these microbial populations could be increased.

The reluctance of growers to leave ozone residuals in the solutions during crop application is understandable; ozone is known to be phytotoxic (Bell and Treshow, 2002; Table 2.4). Having said this, ozone in aqueous solution does not necessarily behave, in terms of phytotoxic potential, the same as ozone in the gas phase. Initial indications were presented by Fujiwara and Fujii (2002), when they investigated the application of aqueous ozone on cucumber for powdery mildew control. In that experiment they applied solutions containing 4.0 mg·L$^{-1}$ aqueous ozone on four occasions. The treatment arrested further development of the powdery mildew but, perhaps more significant, was the lack of phytotoxicity. Little research was published to follow on this observation until Sloan and Engelke (2005) investigated the effects of aqueous ozone on creeping bentgrass. In that study, bentgrass plugs grown in sand-based media were irrigated with ozonated solutions (0.7 – 0.9 mg·L$^{-1}$) for 1 year. These ozone treatments had an initial stimulatory effect, however, it was not sustained. The researchers attribute the initial stimulation to an increase in nutrient mineralization via organic matter oxidation.

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8 ORP is synonymous to Redox Potential, with ORP being the common terminology in N. America.
Although the stimulatory effect was not sustained, there were no indications of phytotoxicity or growth suppression with direct aqueous ozone applications. The concept of applying aqueous ozone to a crop would not appear in the literature again until McDonald (2007). In that work, McDonald applied aqueous ozone solutions to chrysanthemum grown in peat-based media at concentrations up to 1.5 mg·L⁻¹. Once again, there were no indications of phytotoxicity as a result of the direct application of aqueous ozone to the growth substrate.

The removal of aqueous ozone prior to crop distribution is a measure against crop damage; damage that does not appear to occur based on the limited scientific literature available. The following studies were conducted to determine if in fact the removal of aqueous ozone is necessary. Further, if the removal is not necessary, there may be ancillary benefits or applications that can be realized. The final study in this thesis examines one such ancillary application, namely, the control of liverwort.
CHAPTER 3

RESPONSE OF HYDROPONIC TOMATO TO DAILY APPLICATIONS OF AQUEOUS OZONE VIA Drip Irrigation

INTRODUCTION

Increasing competition for diminishing fresh water reserves combined with tightening environmental-based legislative constraints and increasing energy costs have initiated a fundamental operational shift in greenhouse crop production (Johansson et al., 2002; Tognoni et al., 1998; Van Os, 1999; Wallace, 2000). It is no longer feasible, in many production regions, to discard irrigation solutions after a single pass through the production system, be it on social, economic, environmental or legislative grounds. Many greenhouse operators now collect irrigation run-off for reapplication to the crop (Richard et al., 2006). Although this is an efficient use of available resources, it presents problems in terms of deteriorating solution quality including an increased risk of pathogen proliferation resulting from the accumulation and distribution of plant pathogens in the recaptured solution (Hong & Moorman, 2005; Johansson et al., 2002; Van Os, 1999).

Numerous treatment technologies are commercially available to address the various elements of solution deterioration, including but not limited to chlorination, ultraviolet radiation, hydrogen peroxide, filtration, ion exchange resins, heat treatment, and ozonation (Ehret et al., 2001; Hong and Moorman, 2005; Hong et al., 2003, Stewart-Wade, 2011). Of these, ozonation is particularly appealing for study as it is effective for the control of microbial and chemical contamination, while also addressing oxygen deficiencies common in hydroponics (Drew, 1997; Zheng et al., 2007). Furthermore, due to ozone's reactivity with a wide range of cellular constituents there is less chance of pathogen resistance developing to this control measure (Guzel-Seydim et al., 2004). A further benefit of using ozone as opposed to other traditional pesticides is the low potential for residue on or in the crop. Ozone in solution spontaneously reverts to diatomic oxygen (O₂) through complex decomposition mechanisms (Beltrán, 2004; Gottschalk et al., 2000). Aqueous ozone decomposition reactions generate short-lived hydroxyl radicals (OH•) (amongst other radicals and reactive oxygen species), which further increase the

9 The material presented in this chapter is taken in whole or in part from a paper of the same title appearing in Scientia Horticulturae 129:464-471. (2011).
remediation capacity of the treatment technology (Beltrán, 2004; Camel and Bermond 1998). Greenhouse operators that utilize ozonation as a treatment technology typically elect to remove any residual ozone prior to distribution to their crops. The removal, deemed a prudent measure against potential crop damage via ozone off-gassing, is justified under some production scenarios depending on the irrigation system utilized and the ozone concentrations employed (Graham et al., 2009; McDonald, 2007; Sloan and Engelke, 2005). Although the phytotoxicity of ozone has been well established through decades of research on tropospheric ozone enrichment (e.g. photochemical smog) (Fuhrer and Booker, 2003), it should not be assumed that ozone is as phytotoxic in aqueous solution as it is in the gas.

Ozone in aqueous solution is unstable, with a typical half-life in clean water (e.g. tap water) < 20 min (Beltrán, 2004). This instability has a direct influence on phytotoxicity in terms of the actual dose experienced by a plant. Additionally, to enter a plant through normal channels (i.e. stomatal gas exchange) the ozone needs to come out of solution. Once in the gas phase, ozone can pass through the stomatal aperture and re-enter solution at the apoplast water-air boundary inside the sub-stomatal chamber (Heath, 1996). This mass transfer requirement represents an additional resistance to ozone entry and needs to be taken into account when estimating the phytotoxic potential of ozonated irrigation solutions.

The method of application is another important consideration when estimating phytotoxicity of aqueous ozone. Overhead irrigation systems that maintain a residual ozone concentration during application to the crop would have an inherently higher phytotoxic potential than systems that apply the solution directly to the growth substrate. This is due to the increased surface area to volume ratio that occurs upon exit from the emitter (i.e. the stream is converted to droplets), combined with a greater exposure time to the atmosphere surrounding the crop (Fujiwara and Fujii, 2004). Although the off-gassing potential is greater, in previous work it was demonstrated that under controlled experimental conditions, overhead irrigation with residual aqueous ozone could be used on select woody perennial nursery crop species without affecting plant performance (Graham et al., 2009).

Drip irrigation systems apply the solution directly to the growth substrate resulting in little exposure of the solution to the aerial environment. Furthermore, these systems tend to have large drop sizes that do not increase the surface area of the solution to the same degree as overhead irrigation systems. When these considerations are combined with the high ozone demand of the root environment, it is likely that the actual dose experienced by the crop is low. This supposition is supported by previous work, and the work of others that have demonstrated either improved plant growth or no negative impact of root application of aqueous ozone (Graham et al., 2011a; Sloan and Engelke, 2005).
If fertilizer salts are added to the irrigation solution then consideration must also be given to the impact that ozonation will have on the solution nutrient balance. Research has shown that macronutrients are largely unaffected by solution ozonation, while several micronutrients are affected with minor to moderate losses of iron, manganese and boron occurring at ozone concentrations likely to be employed in an irrigation management plan (McDonald, 2007; Ohashi-Kaneko et al., 2009).

This study was undertaken to determine if the removal of residual ozone is justified on the basis of phytotoxicity when the irrigation solution is applied via drip irrigation to hydroponic tomatoes (Lycopersicon esculentum L. cv Matrix F1) grown in mineral wool substrate. The objectives of the study were to: 1) evaluate the growth response of tomato to daily applications of aqueous ozone, and 2) measure the photosynthetic and gas exchange response, as physiological indicators of ozone toxicity, of tomato under daily aqueous ozone applications.

**Materials and Methods**

**Plant Material**

Thiram-treated hybrid tomato seeds (Matrix F1, TmC5OiVFrWi, De Ruiter Seeds Inc. Lakewood, CO) were sown in mineral wool starter plugs (A-Ok Starter Plugs, Grodan Inc., Milton, Ontario, Canada), placed in covered propagation trays and situated in a greenhouse at the University of Guelph (Bovey Research Greenhouse Complex, Guelph, Ont., Canada; 43°33′N 80°15′W). The greenhouse environment control system (Argus Control Systems Ltd., White Rock, British Columbia, Canada) targets were set at 25°C/18°C with a 16 h photoperiod, supplemented by artificial lighting consisting of 400 and 600-W high pressure sodium and metal halide lights respectively. Upon full expansion of the second true leaf (21 d) the plants were selected for uniform height. From these, individual plants were randomly distributed to larger mineral wool cubes (Gro-Blocks Delta 6.5G 42/40, Grodan Inc., Milton, Ontario, Canada). These cubes were then placed on 20x20x7.5cm sections of mineral wool slabs (Gro-Slab Expert 20/75 A2W, Grodan Inc., Milton, Ontario, Canada). The plants were uniformly fertilized every second day with an on-demand soluble fertilizer solution (Plant Products, Brampton, ON. 20-8-20 at 250 mg·L⁻¹; electrical conductivity 2.5 mS; pH 6.0 adjusted with phosphoric acid). This solution was not treated with ozone.
**EXPERIMENTAL DESIGN**

The experiment was set up as a completely randomized design (CRD) with three treatment levels (Control – 0.0 mg·L\(^{-1}\); Low O\(_{3(aq)}\) – 0.5 mg·L\(^{-1}\); high O\(_{3(aq)}\) – 3.0 mg·L\(^{-1}\)). A total of 60 plants per treatment were randomly assigned to locations on two side-by-side growth benches and allowed to establish for 7 d before commencing treatment applications. Three harvests were made in which 20 plants per treatment were randomly selected for destructive analysis. Harvests were made after the 7 d establishment period but prior to treatment initiation (initial), and at days 21 and 49 after treatments commenced (28, 49, and 77 d from sowing respectively). Treatments were applied on a daily basis between 08:30 and 10:30. After the harvest at 21 d, the remaining plants were evenly spaced to reduce crowding as the plants matured.

**AQUEOUS OZONE PREPARATION AND APPLICATION**

Aqueous ozone solutions were prepared at the time of irrigation as described on pages 72-73 (see also Graham et al., 2009), with the exception that the system pressure was lowered to 137.9 kPa from 206.8 kPa.

A sample stream of the treatment solution containing dissolved ozone was drawn off the main feed line upstream from the distribution manifold and directed to a dissolved ozone monitor (Model Q45H, Analytical Technologies Inc., Collegeville, PA.) (Fig. 3.1). The remaining bulk solution was diverted to a bypass until the target dissolved ozone level (Control - 0.0 mg·L\(^{-1}\); Low - 0.5 mg·L\(^{-1}\); High - 3.0 mg·L\(^{-1}\)) was achieved. Once the concentration reached the target treatment level, the bulk solution was redirected to one of three treatment supply lines. Treatments were supplied to manifolds of customized ring-shaped drippers that delivered 250 mL·min\(^{-1}\)±50 mL·min\(^{-1}\) to each plant in the treatment group. Each treatment application was maintained for 5 min, which allowed for an approximate water delivery of 1.3 L·plant\(^{-1}\)·treatment\(^{-1}\). The ozone preparation and distribution system (Fig. 3.1) was able to consistently deliver the target [O\(_{3(aq)}\)] to within ±0.2 mg·L\(^{-1}\). Ambient atmospheric ozone levels were monitored (Model 1004H, Dasibi Environmental Corporation, Glendale, Calif.) to ensure that any potential off-gassing was detected in the bulk atmosphere.
Fig. 3.1: Ozone production and distribution schematic. Oxygen is concentrated from ambient air by removing the nitrogen and water vapour components via a pressure swing absorption (PSA) cycle. The resulting 90-95% $O_2$ gas stream is fed into a variable output corona discharge ozone generator. The ozone is drawn into solution via a venturi injector and mixing loop. Any ozone and oxygen that is not dissolved is vented from the mixing loop and passed over a heated catalyst bed to decompose the ozone. Ozonated irrigation solutions are drawn off the mixing loop into two streams. The first stream enters an aqueous ozone monitor, which provides feedback control for maintaining a fixed ozone concentration. The second stream is diverted to one of three treatment irrigation lines that distribute the solution to the drip-ring emitters around each plant in the treatment.
GROWTH EVALUATION

Leaf Area and Shoot Dry Weight – At each harvest 20 randomly selected plants from each treatment were destructively sampled. Leaves were excised and passed through a leaf area meter (Model LI-3100C, LiCor, Lincoln, Nebraska USA). Following leaf area determination the leaf tissue was placed in paper bags along with all aerial non-leaf tissue. The bags were placed in a drying oven at 65°C until a constant weight was achieved (Sartorius LC12000P, Goettingen, Germany).

Leaf Gas Exchange – Net CO₂ assimilation rate (A), stomatal conductance (gₛ), and internal leaf CO₂ concentration (cᵢ) were measured on the youngest fully-expanded leaf at days [after treatment initiation] 9, 20, 26, 34, 41, and 48, and taken as a physiological indicator of ozone (gas) toxicity. Measurements were made using a portable photosynthesis measurement system (Model LI-6400; LiCor Biosciences, Lincoln, NE). The measurements were made on the plants randomly selected to be in the final harvest to ensure consistency across sampling periods.

Stem Thickness – The diameter of the stem immediately below the sixth leaf node was measured using an electronic digital calliper rule (NSK Max-Cal Max-15, Fowler, Newton, MA) on treatment days 29, 32, 36, 40, 43, and 47.

Fruit – Average fruit number, fresh weight and dry weight were measured on the final 20 plants in each treatment. Due to limited drying space and the time required to dry the mature fruit, the fruit from five randomly selected plants within each treatment were measured instead of the full 20 plants.

Chlorophyll Content Index – The chlorophyll content index was measured weekly (Model CCM-200, Opti-Sciences, Hudson, NH, USA) on the last fully-expanded leaf.

OXYGEN PERSISTENCE IN THE GROWTH SUBSTRATE

Dissolved Oxygen – As a by-product of the ozone production and dissolution process, the irrigation solutions were supersaturated with diatomic oxygen (O₂). Measurements of dissolved oxygen were made before and after passage through mineral wool slabs using a handheld dissolved oxygen meter (Accumet AP64, Fisher Scientific, Canada). Measurements were made on 1.0 L aliquots of untreated tap water, oxygenated water, 0.5 mg·L⁻¹ O₃(aq), and 3.0 mg·L⁻¹ O₃(aq) solutions.
**Aqueous Ozone** – After the final harvest, one litre solutions of 0.5, 2.0, 5.0, 10.0 and 15.0 mg·L⁻¹ O₃(aq) were prepared and passed through the mineral wool slabs. Aqueous ozone concentrations were measured with a handheld colorimeter (DR/890 Portable Colorimeter with ozone reagent HR AccuVac Ampules, Hach Company, Loveland, Colorado, USA) after passage through the mineral wool slab.

**Algae Control**

Algae growth on the surface of the mineral wool was monitored and recorded as a series of digital images. No metric was assigned to the observations beyond a qualitative visual assessment, nor was any attempt made to identify the species present.

**Statistical Analysis**

One-way ANOVA with a Bonferroni post-test, and regression analyses were performed using GraphPad Prism (v 5.0d for Mac, GraphPad Software, San Diego California USA). Unless otherwise stated, all results are reported at a significance level of \( P \leq 0.05 \).

**Results**

**Leaf Area and Shoot Dry Weight**

Leaf area production increased at the highest treatment level (3.0 mg·L⁻¹) throughout the treatment phase of the experiment (Fig. 3.2A). Shoot dry matter accumulation was also greatest at the highest treatment level, but the stimulatory effect was only significant during the first phase of the study with statistical significance being lost by the final harvest period (Fig. 3.2B).
Fig. 3.2: Leaf area development and shoot dry matter accumulation in tomato plants grown under three aqueous ozone irrigation regimes over three harvest periods: A) Leaf area development over 48 days of aqueous ozone application to the mineral wool growth substrate via drip irrigation; B) Dry matter accumulation in the above ground tissue (leaf and stem) over 48 days of aqueous ozone application to the mineral wool growth substrate via drip irrigation. Columns within a harvest period with the same letter above do not differ at $P \leq 0.05$. The values within each bar are the percent change ($\% \Delta$) relative to the control (baseline (BL)) for each harvest period.
**Leaf Gas Exchange**

There were significant differences in net CO$_2$ assimilation rate ($A$), stomatal conductance ($g_s$), or internal leaf CO$_2$ concentration ($c_i$), in any of the ozone treatments applied (Table 3.1), suggesting that ozone gas was not affecting the crop. There were transient improvements in $g_s$ and $c_i$ at the 0.5 mg·L$^{-1}$ treatment level (Day 41 in Table 3.1), but they were not sustained.

**Stem Thickness**

There was a significant increase in stem thickness in the 3.0 mg·L$^{-1}$ treatment compared to the control (Fig. 3.3). The control and 0.5 mg·L$^{-1}$ treatments did not differ.

**Fruit Production**

There were no significant differences in the mean fruit number, fresh weight, or dry weight (Table 3.2).
Table 3.1: Leaf gas exchange summary for each sampling period. Measurements of net photosynthetic assimilation rate (A), stomatal conductance (g_s), and internal CO_2 concentration (c_i) were made on the last fully expanded leaf from each of the 20 plants in the final harvest group in each treatment.

<table>
<thead>
<tr>
<th>SAMPLING PERIOD</th>
<th>TREATMENT</th>
<th>A (µmol m^{-2} s^{-1})</th>
<th>g_s (mmol m^{-2} s^{-1})</th>
<th>c_i (µmol mol^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 9</td>
<td>Control</td>
<td>10.35 ± 1.06 a</td>
<td>599.5 ± 89.1 a</td>
<td>367.8 ± 5.3 a</td>
</tr>
<tr>
<td></td>
<td>0.5 mg·L^{-1}</td>
<td>10.25 ± 0.72 a</td>
<td>635.6 ± 31.4 a</td>
<td>363.6 ± 3.8 a</td>
</tr>
<tr>
<td></td>
<td>3.0 mg·L^{-1}</td>
<td>9.00 ± 0.92 a</td>
<td>542.0 ± 76.4 a</td>
<td>366.2 ± 2.6 a</td>
</tr>
<tr>
<td>Day 20</td>
<td>Control</td>
<td>7.40 ± 0.25 a</td>
<td>712.0 ± 19.7 a</td>
<td>371.8 ± 0.9 a</td>
</tr>
<tr>
<td></td>
<td>0.5 mg·L^{-1}</td>
<td>7.40 ± 0.39 a</td>
<td>714.5 ± 27.2 a</td>
<td>370.5 ± 1.2 a</td>
</tr>
<tr>
<td></td>
<td>3.0 mg·L^{-1}</td>
<td>7.44 ± 0.26 a</td>
<td>695.8 ± 40.3 a</td>
<td>370.0 ± 1.8 a</td>
</tr>
<tr>
<td>Day 26</td>
<td>Control</td>
<td>5.08 ± 0.44 a</td>
<td>364.2 ± 16.6 a</td>
<td>363.7 ± 2.8 a</td>
</tr>
<tr>
<td></td>
<td>0.5 mg·L^{-1}</td>
<td>5.01 ± 0.57 a</td>
<td>328.5 ± 27.7 a</td>
<td>360.5 ± 4.5 a</td>
</tr>
<tr>
<td></td>
<td>3.0 mg·L^{-1}</td>
<td>4.92 ± 0.51 a</td>
<td>314.5 ± 19.6 a</td>
<td>360.8 ± 2.9 a</td>
</tr>
<tr>
<td>Day 34</td>
<td>Control</td>
<td>4.36 ± 0.43 a</td>
<td>566.4 ± 33.4 a</td>
<td>375.9 ± 2.1 a</td>
</tr>
<tr>
<td></td>
<td>0.5 mg·L^{-1}</td>
<td>3.64 ± 0.44 a</td>
<td>502.3 ± 16.4 a</td>
<td>377.7 ± 2.0 a</td>
</tr>
<tr>
<td></td>
<td>3.0 mg·L^{-1}</td>
<td>4.95 ± 0.38 a</td>
<td>534.0 ± 22.4 a</td>
<td>373.5 ± 1.1 a</td>
</tr>
<tr>
<td>Day 41</td>
<td>Control</td>
<td>13.09 ± 0.43 a</td>
<td>485.3 ± 33.0 ab</td>
<td>336.2 ± 3.7 a</td>
</tr>
<tr>
<td></td>
<td>0.5 mg·L^{-1}</td>
<td>12.11 ± 0.42 a</td>
<td>557.3 ± 22.1 a</td>
<td>346.6 ± 2.9 b</td>
</tr>
<tr>
<td></td>
<td>3.0 mg·L^{-1}</td>
<td>12.55 ± 0.43 a</td>
<td>466.7 ± 15.2 b</td>
<td>337.7 ± 2.4 a</td>
</tr>
<tr>
<td>Day 48</td>
<td>Control</td>
<td>14.78 ± 0.57 a</td>
<td>561.3 ± 31.9 a</td>
<td>338.2 ± 2.8 a</td>
</tr>
<tr>
<td></td>
<td>0.5 mg·L^{-1}</td>
<td>15.29 ± 0.24 a</td>
<td>620.4 ± 19.5 a</td>
<td>341.6 ± 1.3 a</td>
</tr>
<tr>
<td></td>
<td>3.0 mg·L^{-1}</td>
<td>14.65 ± 0.51 a</td>
<td>544.5 ± 25.2 a</td>
<td>337.9 ± 2.3 a</td>
</tr>
</tbody>
</table>

a Means (n = 20; ±SEM) within a column and individual sampling period followed by the same letter do not differ at P≤0.05.
Fig. 3.3: Stem diameter measured at the base of the sixth leaf. Individual points are the mean of all twenty plants in the treatment. Vertical error bars are $\pm$SE of the individual means; bounding lines are the 95%CI on the regression. The slopes of the regression lines are not significantly different ($P=0.5831$); elevations are significantly different ($P<0.0001$). The 0.5 mg·L$^{-1}$ treatment level is not presented.
Table 3.2: Fruit production data from the final harvest.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>AVERAGE FRUIT PER PLANT (n=20)</th>
<th>AVERAGE FRESH WEIGHT (grams) (n = 20)</th>
<th>AVERAGE DRY WEIGHT (grams) (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.4 ± 0.5 a</td>
<td>491.6 ± 26.1 a</td>
<td>21.2 ± 1.6 a</td>
</tr>
<tr>
<td>0.5 mg·L⁻¹</td>
<td>12.9 ± 0.5 a</td>
<td>490.5 ± 27.8 a</td>
<td>24.5 ± 4.2 a</td>
</tr>
<tr>
<td>3.0 mg·L⁻¹</td>
<td>13.8 ± 0.6 a</td>
<td>543.8 ± 41.0 a</td>
<td>26.9 ± 7.4 a</td>
</tr>
</tbody>
</table>

a Means within the same column do not differ at $P \leq 0.05$

**CHLOROPHYLL CONTENT INDEX**

There were no differences in the mean chlorophyll content index amongst any of the treatments ($P=0.2585$) on any of the days measured ($P=0.3302$).

**DISSOLVED OXYGEN PERSISTENCE**

Regardless of the initial $[\text{O}_2(\text{aq})]$, the final concentration in all solutions after passage through the mineral wool slab were the same (Table 3.3). All of the supersaturated solutions showed significant reductions ($P<0.0001$) in total $[\text{O}_2(\text{aq})]$ after passage through the mineral wool slab.

Table 3.3: Dissolved $\text{O}_2$ concentrations before and after passage through a 15 cm mineral wool slab.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>$[\text{O}_2(\text{aq})]$ BEFORE</th>
<th>$[\text{O}_2(\text{aq})]$ AFTER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap Water</td>
<td>$3.34^b \pm 0.23 \text{mg·L}^{-1}$ a</td>
<td>$4.75 \pm 0.28 \text{mg·L}^{-1}$ a</td>
</tr>
<tr>
<td>Oxygenated</td>
<td>$16.92 \pm 0.39 \text{mg·L}^{-1}$ b</td>
<td>$4.52 \pm 0.27 \text{mg·L}^{-1}$ a</td>
</tr>
<tr>
<td>0.5 mg·L⁻¹</td>
<td>$18.32 \pm 0.79 \text{mg·L}^{-1}$ b</td>
<td>$4.16 \pm 0.19 \text{mg·L}^{-1}$ a</td>
</tr>
<tr>
<td>3.0 mg·L⁻¹</td>
<td>$18.40 \pm 0.71 \text{mg·L}^{-1}$ b</td>
<td>$4.14 \pm 0.35 \text{mg·L}^{-1}$ a</td>
</tr>
</tbody>
</table>

a Water temperature was 18± 2°C; b – means within the same column followed by the same letter do not differ ($P \leq 0.05$).
**Dissolved Ozone Persistence**

At all the $[O_{3(aq)}]$ tested, there was no detectable residual once the solution passed through the mineral wool slabs (Table 3.4).

Table 3.4: Reduction in $[O_{3(aq)}]$ after a single pass through a 15 cm thick mineral wool block.

<table>
<thead>
<tr>
<th>TEST SOLUTION $[O_{3(aq)}]$</th>
<th>$[O_{3(aq)}]$ IN FLOW THROUGH SOLUTION$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 mg·L$^{-1}$</td>
<td>0.0 mg·L$^{-1}$</td>
</tr>
<tr>
<td>2.0 mg·L$^{-1}$</td>
<td>0.0 mg·L$^{-1}$</td>
</tr>
<tr>
<td>5.0 mg·L$^{-1}$</td>
<td>0.0 mg·L$^{-1}$</td>
</tr>
<tr>
<td>10.0 mg·L$^{-1}$</td>
<td>0.0 mg·L$^{-1}$</td>
</tr>
<tr>
<td>15.0 mg·L$^{-1}$</td>
<td>0.0 mg·L$^{-1}$</td>
</tr>
</tbody>
</table>

$^a$ Solutions consisted of 1 L aliquots

**Algae Control**

A visual inspection of the mineral wool throughout the experiment showed increased areas of clear mineral wool surrounding the drip ring (Fig. 3.4).
Fig. 3.4: Influence of aqueous ozone application on the establishment of algae on the surface of mineral wool growth media. A) Control (no ozone) showing complete coverage; B) 0.5 mg·L⁻¹ treatment showing clear areas immediately below emitter drip points; C) 3.0 mg·L⁻¹ treatment showing a substantial region of clear mineral wool around the emitter.
DISCUSSION

Ozonation as a water remediation tool in greenhouse irrigation management is not a common practice compared to traditional technologies such as slow sand filtration (Ehret et al., 2001; Van Os, 1999). There are numerous factors that have prevented ozonation from gaining wider acceptance in the industry. The cost and complexity, hazard potential, and the perceived need to remove the active compound prior to crop application are offered as arguments against the investment in ozonation as an irrigation management tool (Ehret et al., 2001; Runia, 1994; Stewart-Wade, 2011).

Recent advances in electrochemical ozone production and lower-priced corona discharge ozone generators, have dramatically lowered the capital and operating costs of ozonation systems (Franco et al., 2008; Leong et al., 2008; Onda, 2005). Further, the development of more efficient and automated mass transfer systems has allowed for more compact infrastructure and reduced maintenance making retrofits more feasible (Ozkan et al., 2006; Tizaoui and Zhang, 2010).

Fail-safe mechanisms should be a component of any commercially available water treatment system, irrigation systems being no exception. When operating an ozone-based water treatment system the primary concern, in terms of system failure, are leaks in the ozone feed-gas and off-gas lines. Ozone leaks pose a significant hazard to employee (Government of Canada, 2012; Leong et al., 2008) and crop health. Reliable ozone gas sensors are readily available (e.g. personal dosimeters) and should be included in any ozonation system; however, the cost of reliable monitoring systems can be a significant percentage of the overall system cost and typically require a moderate level of maintenance to ensure proper functionality. Lower cost sensing systems tend to suffer from reliability issues and, in the author's experience, are not technically mature or reliable enough to justify use in a fail-safe system, although advances are being made.

The presented data support the retention of residual ozone in the irrigation solution during distribution to the crop; however, as the concentration of ozone in solution increases so too does the driving force behind off-gas evolution. This will result in unacceptable amounts of ozone entering the gas phase in the immediate vicinity of the crop (Sandermann, 1996). Ozone exposure under this scenario would fall into the acute rather than the chronic exposure regime typical of prolonged periods of smog exposure. There is on-going debate surrounding the differences in mechanisms and the extent of damage under acute versus chronic ozone exposures (Chen et. al., 2009). It is difficult then to speculate on the risk or extent of crop damage beyond those application regimes examined in the current study, although recent studies indicate that high $O_{3\text{aq}}$ concentrations (i.e. 15 to 20 mg·L$^{-1}$) can be employed if
the exposure frequency and duration is low (Graham et al., 2011a; Appendix 2). The long-term efficacy, in terms of pathogen control, of low frequency applications is, however, questionable.

Growers are in need of treatment technologies that will address contamination throughout their irrigation system, from cistern to emitter and back again. The removal of ozone prior to distribution severely limits the potential of the treatment as a system wide solution and adds complexity and cost to the overall set-up. McDonald (2007) examined aqueous ozone application to peat-based growth substrate in chrysanthemum production. In that study, there were no indications of phytotoxicity due to the media application of aqueous ozone. Sloan and Engelke (2005) applied aqueous ozone to Agrostis stolonifera L. and again there were no symptoms of phytotoxicity. The data presented here supports the results of these earlier studies and clearly demonstrated that the removal of residual ozone from the treated solution is not a requirement in drip irrigation systems utilizing mineral wool as a growth substrate for hydroponic tomato production. In no instance was a reduction in plant productivity observed (Table 3.1; Table 3.2). On the contrary, improvements were realized at the highest ozone [3.0 mg·L⁻¹] treatment level (Fig. 3.2; Fig. 3.3). It is difficult to say if the presence of ozone is the main cause or one of several contributing factors to the enhanced growth response.

A by-product of ozone production and dissolution is significantly increased dissolved oxygen due to the 90-95% O₂ gas used in the generation process. Zheng et al., (2007) demonstrated that supersaturated solutions up to 30 mg·L⁻¹ dissolved O₂ can improve production, albeit only slightly. Table 3.3 shows that the solutions applied in this study were, in fact, supersaturated (saturation at 20°C is ~7.6 mg·L⁻¹), which may have contributed to the overall improvement at the 3.0 mg·L⁻¹ treatment level; however, the effect should have been consistent across all the treatments as the dissolved oxygen concentrations were elevated in all treatments (Table 3.3) in an attempt to isolate the effects of ozone. If an assumption is made that there are no interactive effects of elevated O₂ and O₃, then it would seem that the ozone is having a stimulatory effect, at least during the early growth phase (Fig. 3.2). This conjecture is supported by the work of Sloan and Engelke (2005) who demonstrated a similar early stimulatory effect of aqueous ozone drenches.

Algae are known to have a detrimental influence on plant productivity in greenhouse systems (Chase and Osborne, 1984). A substantial population of algae can clog emitters, compete for nutrients, limit oxygen diffusion into the root zone, and in some cases produce plant toxins (Carrow, 1996; Schwarz and Gross, 2004). Algal mats (on substrate surfaces) also provide habitat for plant pests such as fungus gnats and shore flies (Scatella spp.), which can damage root systems and act as pathogen vectors,
respectively (El-Hamalawi, 2008; Vanninen and Koskula, 1998). Delivery of ozone directly to the mineral wool growth substrate had a clear and negative influence on the establishment of algae at the substrate surface. This reduced algae cover may have contributed to the improved plant performance under the highest ozone treatment level.

Algae are difficult to control in greenhouse hydroponics (Chase and Osborn, 1984) and the observation that the ozone treatments appear to maintain clear zones on the mineral wool without any phytotoxic effects on the crop expands the applicability of the technology for greenhouse drip irrigation system management. This study was not designed to examine the influence of ozone on algae establishment. Further studies should be conducted to quantify the response noted here.

Ozone off gassing could occur at two points after the solution leaves the emitter. The first opportunity to realize off gassing issues is between the emitter and the growth substrate. In this study the emitters were in direct contact (or very close to) with the mineral wool, leaving little opportunity for ozone gas to escape at the discharge point. The second point where off gassing could be a problem would be in the run-off water if residual ozone remained. The bulk atmospheric ozone concentrations in the greenhouse zone did not rise above ambient levels during the study (data not shown), suggesting that under the conditions employed in this study off gassing was not a major concern. Table 3.4 confirms that the run-off water was also not a significant source of ozone off-gas. Even at high ozone concentrations (15 mg·L\(^{-1}\)), there was no residual ozone remaining in the solutions after passage through the mineral wool slab. This suggested that ozone was completely consumed by root zone constituents, which may include pathogens as well as other potentially detrimental compounds. Further, there were no differences in measured photosynthesis and stomatal conductance between the treatments. If biologically relevant amounts of ozone had come out of solution, one would expect a difference in these parameters, as ozone is known to affect photosynthesis and stomatal conductance (Heath, 1996).

The oxidation of these root zone constituents may have been a further contributing factor to the enhanced plant growth observed. Sloan and Engelke (2005) applied aqueous ozone to creeping bentgrass (Agrostis stolonifera) plugs and observed an initial increase in productivity, as well as an increase in nutrients in the leachate collected. They attributed this to an ozone-enhanced mineralization of root zone organic material. Mineral wool inherently has less organic matter than the system used by Sloan and Engelke. Therefore, the effect should be less influential on productivity in the present study. The relative amount of root material within the media in each of the studies is not known and may balance out the intrinsic organic matter differences in the media used. Further studies that characterize
the algae populations, quantify the level of control, and examine the interception of water and nutrients are required to evaluate this potential contribution to overall productivity.

The results presented demonstrate that aqueous ozone, delivered via drip irrigation, increases productivity; however, the net photosynthetic assimilation rate data did not show any differences between the treatments (Table 3.1). This discrepancy cannot be overlooked, as the only way to have an increase in accumulated dry matter (Fig. 3.2 B) is to have a greater net carbon assimilation rate. This highlights a drawback of the system used (LI-6400, LiCor Biosciences, Lincoln, NE) to measure the photosynthetic rate. The LI-6400 was used to measure a section of the last fully expanded leaf on each plant. Although a powerful tool, this system does not necessarily reflect the overall photosynthetic capacity of the plant, given that the measure is made on a single leaf. This is likely the source of the discrepancy observed. Further studies that utilized net carbon exchange chambers capable of whole-plant photosynthetic evaluation are required to resolve this outstanding issue.

**CONCLUSIONS**

The prudence exercised through the removal of residual aqueous ozone from treated irrigation solutions prior to distribution to the crop is not justified in terms of potential negative impacts on plant productivity when using drip style irrigation systems. On the contrary, this study supports the conclusion that low (e.g. 0.5 mg·L\(^{-1}\)) to moderate (e.g. 3.0 mg·L\(^{-1}\)) residual ozone solutions applied directly to a mineral fibre growth substrate improves several key tomato performance metrics. In no instance was the ozone treatment detrimental to productivity. The benefits of retaining a residual ozone concentration during distribution to the crop, in terms of irrigation system maintenance and hygiene, as well as water use efficiency, can now be realized in drip hydroponic tomato culture.
CHAPTER 4

CLOSING IN ON UPPER LIMITS FOR ROOT ZONE AQUEOUS OZONE APPLICATION IN MINERAL WOOL HYDROPONIC TOMATO CULTURE

INTRODUCTION

Ozone-based technologies and applications servicing the agriculture and agri-food sectors continue to experience a slow but steady growth (Sopher et al., 2002). Current and emerging applications include the extension of post-harvest shelf life in commodities as diverse as wheat (Wu et al., 2006) and cut roses (Robinson et al., 2009), soil fumigation (Larson, 2002), and the remediation of irrigation water in greenhouse and nursery operations (Bourbos and Barbopoulou, 2005; Ehret et al., 2001; McDonald, 2007; Stewart-Wade, 2011).

Amongst the aforementioned applications, the treatment of irrigation water is perhaps the most transformative when recognizing that both the quality and quantity of available water resources is a major limiting factor in crop production. Accelerating population growth and shifting climactic regimes will intensify the pressure on global fresh water supplies, in many cases to the point of acute scarcity (Seckler et al., 2001). Many cropping systems will not be sustainable without comprehensive changes to current irrigation practices.

Greenhouse and nursery operators generally recognize this resource shortfall and the role that they play in it. As a whole, the industry has been slowly modifying practices and looking to new technologies and research to facilitate this adaptation (Richard et al., 2006; Tognoni et al., 1998). The capture and reuse of irrigation drainage is perhaps the most significant management shift in this regard. Under this strategy, irrigation runoff is intercepted using impermeable membranes, troughs, etc., so as to retain water within the system rather than discharging to the surrounding environment.

Although recycling irrigation water in this fashion is an efficient use of water resources, it does present problems in terms of water quality maintenance including nutrient imbalances, chemical

10 The material presented in this chapter is taken in whole or in part from a paper of the same title appearing in *Scientia Horticulturae*. 143:151-156. (2012).
contamination, and pathogen proliferation. Nutrient imbalances arise when crops take up nutrients in ratios differing from the ratios at which nutrients are supplied. Over successive recirculation cycles, this imbalance can lead to nutrient deficiencies and/or toxicities. Chemical contamination can result from numerous sources ranging from compounds leached from irrigation system hardware to atmospheric deposition and pesticide application. Depending on the nature and concentration, these contaminants may render the water unsuitable for further irrigation.

Notwithstanding the importance of the preceding concerns, it is the threat of pathogen proliferation that dominates grower decisions regarding irrigation water recycling (Richard et al., 2006). Root infecting pathogens have a demonstrated capacity to spread via contaminated irrigation solutions (McDonald, 2007; Stewart-Wade, 2011), and it is this threat that has slowed the adoption of irrigation systems that recycle run-off water (Richard et al., 2006).

A clear solution to this problem is the incorporation of remediation technologies and management practices aimed at reducing pathogen pressures. Stewart-Wade (2011) provided a comprehensive review of established and emerging technologies used for the control of pathogens in irrigation systems.

Ozonation, the dissolution of ozone gas into solution, is one technology available for use in the remediation of irrigation water. In a typical greenhouse installation, irrigation water is treated and then stored in holding tanks to allow the ozone to decompose. Alternatively the solution is passed through filters that remove or decompose the ozone prior to crop application. This is a pertinent practice, as ozone gas is widely known to be one of the major phytotoxic constituents of photochemical smog.

However, eliminating dissolved ozone from the solution prior to distribution negates any possibility for system wide pathogen control. Recent studies suggest aqueous ozone is not as significant a risk, in terms of phytotoxicity, as would be expected based on available gas phase data (Graham et al., 2011b; Graham et al., 2009; McDonald, 2007; Sloan and Engelke, 2005). Paradoxically, in some cases the application of aqueous ozone can improve overall plant productivity (Sloan and Engelke, 2005).

In a previous study, it was demonstrated that daily applications of aqueous ozone in hydroponic mineral wool tomato culture could improve plant productivity (Graham et al., 2011b; Chapter 3). In that study, improved plant performance was observed even at the highest aqueous ozone application level employed (3.0 mg·L⁻¹). This is promising in terms of developing expanded roles for aqueous ozone in irrigation management, but if ozone is to remain in the solution during distribution to the crop then it is...
imperative that the upper tolerance limits be resolved so that the systems can be run at peak pathogen control efficacy, while minimizing any potential for crop damage.

The objectives of the presented study were to: 1) determine if enhanced oxygenation, as a by-product of the ozonation process, influenced productivity; 2) determine an upper limit for aqueous ozone application under the conditions examined; and 3) determine the degree to which aqueous ozone penetrated the mineral wool growth substrate.

**MATERIALS AND METHODS**

**PLANT MATERIAL AND HORTICULTURAL MANAGEMENT**

Thiram treated hybrid tomato (*Solanum lycopersicum*) seeds (Matrix F1, TmC5OiVFrWi, De Ruiter Seeds Inc. Lakewood, CO) were sown in mineral wool starter plugs (A-Ok Starter Plugs, Grodan Inc., Milton, Ontario, Canada), placed in covered propagation trays and situated in a greenhouse at the University of Guelph's Bovey Research Greenhouse Complex (Guelph, Ont., Canada; 43°33'N 80°15'W). Environment control (Argus Control Systems Ltd., White Rock, British Columbia, Canada) targets were set at 25°C/18°C day/night with a 16-h photoperiod, supplemented by artificial lighting consisting of 600 and 400-W high pressure sodium and metal halide lights respectively. Upon full expansion of the second true leaf (21 d), the plants were selected for uniform height, randomly distributed to larger mineral wool cubes (Gro-Blocks Delta 6,5G 42/40, Grodan Inc., Milton, Ontario, Canada), and randomly assigned to positions on the grow benches. The plants were uniformly fertilized every second day with an on-demand soluble fertilizer solution (Plant Products 20-8-20; 250 mg·L⁻¹; electrical conductivity 2.5 mS; pH 6.0 adjusted with phosphoric acid). The crop was allowed to acclimate (7 d) prior to treatment initiation. The plants were 28 d old at treatment initiation.

**AQUEOUS OZONE PRODUCTION AND DELIVERY**

Aqueous ozone solutions were prepared at the time of irrigation and distributed to the plants within a given treatment group [0.0 mg·L⁻¹ (O₂ saturated control); 0.0 mg·L⁻¹ + O₂ (i.e. O₂ supersaturated control); 2.0 mg·L⁻¹; 4.0 mg·L⁻¹; 6.0 mg·L⁻¹] as described by Graham et al. (2011b). The oxygen supply was removed from the dissolution system during preparation of the saturated control treatment (0.0 mg·L⁻¹) solution so that only ambient air was mixed with the irrigation stream. The oxygen supply was reconnected for all other treatments including the supersaturated control (0.0 mg·L⁻¹ + O₂; 16 mg·L⁻¹
nominal oxygen concentration) treatment. The second control (0.0 mg·L⁻¹ + O₂) was included to allow for isolation of ozone effects.

Treatments were supplied to irrigation manifolds of customized ring drippers that delivered 200±50 mL·min⁻¹ to each plant in the treatment group. Each treatment application was maintained for 20 min during the first nine days of the study. After nine days, the duration was reduced to five min applications to reduce the demands on the deionized water system and to reduce the potential for ozone off gassing. The five min watering duration provided a volume of water approximately twice that of the mineral wool block (in terms of water volume : mineral wool volume) and minimized the off gassing at the highest aqueous ozone level. Ambient atmospheric ozone levels were monitored (GasSens, Analytical Technologies Inc., Collegeville, PA.) to ensure that any significant (hazardous) off gassing was detected in the bulk atmosphere.

EXPERIMENTAL DESIGN

GREENHOUSE TOMATO PRODUCTION EXPERIMENT

The experiment was arranged as a randomized complete block design (RCBD) (n = 12) with sub-sampling. There were five treatments: (1) 0.0 mg·L⁻¹ (saturated control – nominal dissolved oxygen concentration between 8-10 mg·L⁻¹ depending on daily solution temperatures); (2) 0.0 mg·L⁻¹ + O₂ (supersaturated control – nominal dissolved oxygen concentration of 16 mg·L⁻¹); (3) 2.0 mg·L⁻¹; (4) 4.0 mg·L⁻¹; (5) 6.0 mg·L⁻¹ in each of twelve blocks with eight sub-sample plants per treatment level in each block. All the treatments containing ozone also had a nominal O₂ concentration of 16 mg·L⁻¹. A row of buffer plants around each bench was incorporated into the design to minimize edge effects. An initial harvest was conducted at the time of plant transfer to the treatment system. Two additional harvests (second and final harvest) were conducted at days 14 and 29 after treatment initiation, during which four plants (sub-samples) were randomly selected from each treatment in each block.

MINERAL WOOL CONTACT EXPERIMENTS

To determine the ozone removal capacity of the mineral wool, which regulates the degree to which aqueous ozone penetrates into the root zone, a system was needed that would allow for controlled and repeatable ozone applications to the substrate. A system was developed in which 2 cm thick mineral wool disks, with a diameter of 5.1 cm, were fitted into the end of a clear PVC pipe with an internal diameter matching that of the disks. The mineral wool disks were cut from the top 2 cm of mineral wool
slabs (Gro-Top Master, Grodan Inc., Milton, Ontario, Canada). Only the top 2 cm of the slabs were used to ensure relative uniformity in the density of the disks. Aqueous ozone solutions were then introduced into the pipe and allowed to drain through the mineral wool disk(s). The initial ozone concentrations were determined with an in-line aqueous ozone monitor (Q45H, Analytical Technologies Inc., Collegeville, PA) that was calibrated to match the concentration at the sample application port. The calibration reference was the indigo method (AccuVac, HACH Company, Loveland, CO). Samples were collected after passage through the mineral wool disks and the aqueous ozone concentration was measured using the indigo method previously used to calibrate the in-line monitor.

To ensure that only the inherent ozone destruction capacity of the mineral wool was evaluated, and not contributions from transient contaminants, it was necessary to: 1) rinse out any soluble organic or inorganic compounds (e.g. wetting agents added during manufacture), and 2) ensure there was little or no organic material in the mineral wool. To achieve this, the disks were soaked in deionized water for one hour and then drained. This procedure was repeated three times to ensure complete rinsing. The disks were then briefly (<10 min) transferred to a flow-through bath that was fed with a high concentration aqueous ozone solution (>7.0 mg L⁻¹) using the aqueous ozone system described previously (Graham et al., 2011a). In addition to meeting the previously stated conditions, the ozone pre-treatment also ensured that any ozone removal capacity observed during testing would be an inherent characteristic of the mineral wool rather than a transient ozone demand.

Mineral Wool Experiment 1 (MWE-1)

In the first experiment, 250 mL aliquots of aqueous ozone (pH 7.2; 12.5 C) were poured through successively thicker sets of mineral wool plugs that were pre-wetted with deionized water. Fresh sets of disks were used for each test (n=3). The treatment regime consisted of five nominal concentrations of aqueous ozone (0.5, 1.5, 3.0, 4.0, 6.0 mg·L⁻¹). Disks were added until no residual ozone was detected in the leachate. In this experiment, the ratio of the volume of solution versus volume of the mineral wool was (relatively) low, so a minor correction was made for dilution effects based on plug flow fluid dynamic principles and empirical results of pre-trial testing. Briefly, it was assumed that the water passing through the mineral wool disk moved as a uniform plug. The plug that was initially held in the mineral wool was assumed to be free of ozone and would dilute the collected sample. The final concentration accounts for this minor dilution.
**Mineral Wool Experiment 2 (MWE-2)**

Based on the results of the first experiment, a second experiment was conducted to determine at what point the aqueous ozone overwhelmed the ozone removal capacity of the mineral wool, as well as to determine how much and how far the ozone penetrated the growth substrate. In this study, a continuous stream of aqueous ozone (0.5, 1.5, 3.0, 6.0 mg·L\(^{-1}\)) was delivered to the top of the mineral wool disk(s) at a rate of one L·min\(^{-1}\). The leachate was periodically sampled and the residual ozone measured via the indigo method. In this experiment, the ratio of the volume of solution applied versus the volume of the disks was high (i.e. 25 – 500 times the volume of the mineral wool disks) and a dilution factor was not required.

**Growth Evaluation**

**Leaf Area and Shoot Dry Mass**

At each harvest, four plants were randomly selected from each treatment in each of the twelve blocks and destructively sampled. Leaves were excised and passed through a leaf area meter (LI-3100C, LiCor, Lincoln, Nebraska USA). Following leaf area determination, the leaf tissue was placed in paper bags along with all aerial non-leaf tissue. The bags were placed in a drying oven at 65°C until a constant weight was achieved (Sartorius LC12000P, Goettingen, Germany) in a representative sample, at which point the dry mass was determined for each plant.

**Root Dry Mass**

Removing root material from mineral wool is a difficult task and often cannot be justified on a time requirement basis, particularly with the number of samples that were harvested at each sampling period (240 plants). An estimation of the root dry mass was obtained for each mineral wool block by weighing each block prior to the experiment. The mass of the clean block was then subtracted from the final dry mass of the block plus root materials to give an estimate of the root dry mass. It is conceded that some additional error is introduced due to salt and algae contributions to the total mass. The contributions of these two factors were considered small and treated as a systematic offset.
**STATISTICAL ANALYSIS**

A two-way ANOVA with a Bonferroni post-test was utilized to evaluate differences between the treatments (v 5.0d for Mac, GraphPad Software, San Diego California USA). Specific treatment contrasts presented in Table 1 were computed using SAS (procGLM, SAS 9.13, Cary, NC).

**RESULTS**

**SHOOT ANALYSIS**

Both leaf area (LA) (Table 4.1; Fig. 4.1) and total shoot dry weight (SDW) (Table 4.1; Fig. 4.2) were lower at day 14 for plants receiving the 4.0 mg·L⁻¹ and 6.0 mg·L⁻¹ treatments, as compared to the air-only control. This interval covers the extended watering period of the trial. Plants harvested at day 29 showed no suppression at 4.0 mg·L⁻¹, but growth was still suppressed at the 6.0 mg·L⁻¹ treatment level.

**ROOT ANALYSIS**

Root dry weight (RDW) measurements are summarized in Fig. 4.3. The RDW accumulation was suppressed at both the second and final harvests at the 6.0 mg·L⁻¹ treatment level. Unlike the aerial plant organs, root development was not impacted at the 4.0 mg·L⁻¹ treatment level. The initial harvest RDW is not included as the root development was too small at that early stage of development and the root mass was lost in the overall variability of the measurement protocols.

**OZONE PENETRATION ANALYSIS**

*MWE-I*

No residual ozone was detected after passage through a single 2 cm mineral wool disk until the 3.0 mg·L⁻¹ treatment level (Fig. 4.4). Although residual ozone was detected, it was small (1%) compared to the inlet concentration. Even at 6.0 mg·L⁻¹, there was only 6.6% of the ozone remaining in the solution after contact with the mineral wool (Fig. 4.4).
Table 4.1: Contrast summary for specific plant growth parameters at the second and final harvests.

| PARAMETER | LEAF AREA | | SHOOT DRY WEIGHT | | ROOT DRY WEIGHT | |
|-----------|-----------|-----------|-----------|-----------|-----------|
|           | Second Harvest (day 14) | Final harvest (day 29) | Second Harvest (day 14) | Final harvest (day 29) | Second Harvest (day 14) | Final harvest (day 29) |
| 0 vs. $0_{+}\text{O}_2$ | 0.3069$^b$ | 0.2586 | 0.5393 | 0.3396 | 0.9320 | 0.1444 |
| $0 + 0_{+}\text{O}_2$ vs. 2, 4, 6 mg·L$^{-1}$ | 0.0013 | 0.2907 | 0.0022 | 0.2036 | 0.0071 | 0.2230 |
| $0 + 0_{+}\text{O}_2$ vs. 2 + 4 mg·L$^{-1}$ | 0.0237 | 0.7064 | 0.0333 | 0.7920 | 0.0350 | 0.9253 |
| $0 + 0_{+}\text{O}_2$ vs. 6 mg·L$^{-1}$ | 0.0004 | 0.0043 | 0.0006 | 0.0182 | 0.0084 | 0.0055 |

$^a$ – contrasts are orthogonal
$^b$ – $P$-values as obtained from the procGLM (SAS) analysis
Fig. 4.1: Leaf area development in mineral wool, hydroponic-cultured, tomato plants under five aqueous ozone treatments over three harvests. The initial harvest was conducted after establishment but prior to treatment initiation (i.e. 28 d). Columns within each harvest period with the same letter appearing above them do not differ at $P \leq 0.05$. Error bars are the SEM (n = 12).
Fig. 4.2: Total shoot dry matter accumulation in mineral wool, hydroponic-cultured, tomato plants under five aqueous ozone treatments over three harvests. The initial harvest was conducted after establishment but prior to treatment initiation (i.e. 28 d). Columns within each harvest period with the same letter appearing above them do not differ at $P \leq 0.05$. Error bars are the SEM (n = 12).
Fig. 4.3: Root dry matter accumulation in mineral wool, hydroponic-cultured, tomato plants under five aqueous ozone treatments over three harvests. Columns within each harvest period with the same letter appearing above them do not differ at $P \leq 0.05$. Error bars are the SEM (n = 12).
Fig. 4.4: Aqueous ozone removal/decomposition due to passage through mineral wool. Aqueous ozone aliquots of 250 mL were passed through mineral wool disks (insert) with an approximate volume of 40-mL. Error bars are the SEM (n = 3). Inset: Sample of the mineral wool disks utilized in the study. The numbers in or beside each column are the actual aqueous ozone concentrations as measured by the indigo method.
Ozone residuals were detected at all the concentrations tested after 1-L of solution had passed through 2-cm of mineral wool (Fig. 4.5). Overall, there is a general trend of increased residual aqueous ozone as the volume of solution passed through the disks (regardless of thickness) increased; however, in no case tested was it observed that the ozone removal capacity of the mineral wool was completely overwhelmed (Fig. 4.5).

**DISCUSSION**

Enhanced solution oxygenation is concomitant with ozonation. Depending on system configuration and irrigation protocols utilized, the irrigation solution will be fully oxygen saturated or even supersaturated at the time of delivery to the crop. Many greenhouse and nursery operators have invested in aeration systems to ensure sufficient oxygen in the root zone. Low oxygen levels in the root zone have well-established negative impacts on plant productivity (Drew, 1997; Morard et al., 2000). On the other hand, data on the effects of super saturation are sparse, but there is some evidence that productivity can be marginally enhanced (Zheng et al., 2007).

With the potential for increased productivity due to increased oxygenation as a by-product of the ozonation process, a double control was employed in this study. A fully aerated control (saturated) and an enhanced oxygenation control (supersaturated) were used to resolve the direct effects of ozone and the potential effects of enhanced oxygenation. In the current study, enhanced oxygenation did not improve productivity relative to the fully aerated control (Table 4.1; Fig. 4.1 – 4.3); any impact on productivity is attributed to the effects of aqueous ozone. Although there were no differences related to oxygenation observed in this study, the separation in terms of oxygen concentration between the saturated and supersaturated solutions were narrow relative to the work of Zheng et al., (2007), and it is difficult to make direct comparisons with that study in which improved productivity was demonstrated. Although valid in the present context, the results on the oxygenation effect shown in this study should not be considered definitive. The stimulatory effects demonstrated by Zheng et al (2007) were only realized at dissolved oxygen concentrations above 20 mg·L$^{-1}$, but below 40 mg·L$^{-1}$. Achieving these levels in commercial systems would require specialized equipment beyond that of typical ozonation systems, further adding to the system costs. The minimal productivity improvements demonstrated by Zheng et al. (2007) would not likely off-set the added costs, but follow-up studies would confirm this.
Fig. 4.5: Aqueous ozone removal/decomposition after increasing volumes have passed through mineral wool plugs of various thicknesses. A) aqueous ozone residual after 1, 2, 5, 7 and 10 L of 0.5 mg L$^{-1}$ aqueous ozone solution had passed through a 2 cm and 4 cm thick mineral wool plug; B) aqueous ozone residual after 1, 2, 5, 7 and 10 L of 1.5 mg L$^{-1}$ aqueous ozone solution had passed through a 2, 4, and 6 cm thick mineral wool plug; C) aqueous ozone residual after a range of solution volumes at 3.0 mg L$^{-1}$ had passed through a 2, 4, 6, and 8 cm thick mineral wool plug; D) aqueous ozone residual after 1, 3, 5, and 7 L of 6.0 mg L$^{-1}$ aqueous ozone solution had passed through a 2, 4, 6, and 8 cm thick mineral wool plug. The top section of each column (dark/green) is the percent ozone removed from the solution (numeric value is given within the column). The lower section of the column is the percent residual remaining in the solution after passage. The ozone consumption ratio is the ratio of ozone remaining to the ozone removed.
During the first phase of this study (days 1-9) the irrigation period was set at 20 minutes, which was excessive in terms of water application. Further, at the highest treatment level (6.0 mg·L\(^{-1}\)) ozone off-gas could be detected by the end of the irrigation cycle. Further testing would indicate that approximately 30% of the ozone does survive passage through a full thickness mineral wool block when excessive volumes and high concentrations are employed (Fig. 4.4 – 4.5; 4 – 5 L applied at 6.0 mg·L\(^{-1}\)). The 5 min irrigation period employed for the remainder of the study did not result in any off gassing as no ozone survived passage through the mineral wool at this volume (1 L) (Fig. 4.5).

Productivity suppression observed at 4.0 mg·L\(^{-1}\) during the second harvest, which included all of the high irrigation period and only a few days at the reduced watering levels, was not carried through to the final harvest (Table 4.1; Fig. 4.1 – 4.3). This suggests a recovery at 4.0 mg·L\(^{-1}\). Recovery to the point of no statistical difference by the final harvest implies that the growth rate was increased relative to the controls. Although it is only conjecture based on the data available in this study, the improved growth is at least partially supported by a previous study (Graham et al., 2011b).

Typical irrigation events in a commercial greenhouse would target a run-off of 20-30% to ensure proper media wetting. In the first mineral wool experiment (MWE-1) the run-off ratio (solution volume to mineral wool volume) was 6.25 : 1 (625% runoff), which is well above the commercial norm. Regardless, little or no ozone remained in the leachate after passage through 2 cm of mineral wool (Fig. 4.4); with no ozone remaining at any of the treatment levels when 4 cm of mineral wool were used (data not shown). Any direct effects of ozone (e.g. algae suppression, nutrient mineralization, root damage/oxidation etc.) would only be realized in the upper regions of the mineral wool – root complex under typical irrigation events. This is not to say that secondary influences, such as formation of other reactive oxygen species that do penetrate further into the mineral wool, are negligible. The current study did not examine these potential effects, but it is a vital component of the story and needs to be addressed in future studies.

In the second mineral wool experiment (MWE-2), a continuous flow of aqueous ozone solution was allowed to pass through the mineral wools disks. The intent was to determine when ozone overwhelmed the removal capacity of the mineral wool. The results are summarized in Fig. 4.5 and indicate that only at the highest ozone concentrations did enough ozone survive passage through a full thickness mineral wool block (8-cm) to register a residual in the leachate after three liters had passed through. This represents a run-off ratio of 18.75:1 or 1875% before ozone breakthrough occurs. Based on this, it is reasonable to conclude that ozone will not be present in the leachate in typical commercial systems, and
so the potential for off-gassing will be reduced. It is also reasonable to conclude that the removal capacity of the mineral wool will remain intact for the life of the crop, although further study is required to confirm this.

Mineral wool is composed primarily of silica and aluminum oxides with numerous other metal and transition metal oxides also appearing in the mix (McConnell, 2000). Some of these oxides are known to act as ozone decomposition catalysts (Heisig et al., 1997; Thomas et al., 1997). Combined with the high surface area of the medium supporting these catalysts, it is not surprising that so much of the aqueous ozone was removed or consumed during passage through the fibres (Fig. 4.4 – 4.5). Given this catalytic activity, spent mineral wool may prove to be an inexpensive off-gas decomposition option for growers looking to shave some of the cost off of their systems.

Ozone decomposition on metal oxide catalysts can generate reactive oxygen species (ROS; e.g. superoxide) (Dhandapani and Oyama, 1997). The movement and activity of these ROS within the root zone is currently unknown, but as indicated earlier the fate of these ROS could be a significant component of this story and needs further research.

The production improvements observed previously (Graham et al., 2011b) were not observed during the present study; rather the treatments (except 6.0 mg·L⁻¹) did not differ from the controls. This may be a result of overall plant age, as the plants in this study had not yet reached fruiting stage, whereas the plants in the previous study were mature and in full fruit production. Improvements in productivity may be small and incremental, discernable only after plants reach maturity. The initial excessive exposure may have also suppressed overall improvements, although recovery was observed in the 4.0 mg·L⁻¹ treatment (Table 5.1; Fig. 4.1 – 4.3).

The primary goal of the present study was to identify an upper threshold for daily direct application of aqueous ozone in mineral wool hydroponic tomato culture using drip irrigation. In all the agronomic parameters measured, there was suppression in productivity at the 6.0 mg·L⁻¹ treatment level. Initial overexposure may have contributed to the observed suppression. Regardless, there was initial suppression at 4.0 mg·L⁻¹ and continued suppression throughout the study at 6.0 mg·L⁻¹. It would be prudent to set an upper limit threshold below any level that is at all questionable until further evidence is available; therefore the author’s suggest a conservative upper limit of 3.0 mg·L⁻¹ in mineral wool hydroponic tomato production utilizing drip irrigation, a level previously demonstrated to improve
productivity (Graham et al., 2011b). Beyond 3.0 mg·L⁻¹ the potential for phytotoxicity increases in daily application scenarios.

The application strategy employed will dramatically influence the concentration of aqueous ozone utilized. Clearly, if aqueous ozone were used to clean the irrigation system between crops, much higher levels would be employed, as there is no concern over crop damage. Other scenarios could see periodic applications at high concentrations, followed by routine applications at lower concentrations. In this scenario, the high concentration application could be timed to coincide with minimal gas exchange rates in the crop, such as a dark period irrigation cycle (i.e stomates largely closed at night).

**CONCLUSIONS**

Aqueous ozone can be applied to tomato plants grown in hydroponic mineral wool culture employing drip irrigation, but there are caveats on its use. Based on the results of the current study and previous work (Graham et al., 2011b), a conservative safe upper operating limit is 3.0 mg·L⁻¹ at the point of discharge, applied once per day. The current study was limited to one aqueous ozone irrigation event per day. Commercial irrigation frequencies however are much higher and as such the aqueous ozone exposure would be greater. Further research is needed, and circumspection is required, if this guideline is to be used in a setting where the irrigation events that incorporate aqueous ozone exceed one event per day.
CHAPTER 5

PHYTOTOXICITY OF AQUEOUS OZONE ON FIVE CONTAINER-GROWN NURSERY SPECIES11

INTRODUCTION

In many of the world’s largest greenhouse and nursery production regions irrigation water quality and supply have become significant operational barriers. Increasingly restricted water supplies, coupled with the perennial threat of emerging and existing disease and pest vectors, present significant obstacles to achieving optimal nursery and greenhouse production (Hong and Moorman, 2005; Johansson et al., 2002). These production barriers are exacerbated by shifting consumer and legislative demands that limit the ability of production managers to deal with resource scarcity and pest issues (Province of Ontario, 1990; Yiridoe et al., 2005). Consumers are becoming more conscious of chemical and resource use, while evolving government regulations will significantly restrict or alter traditional water use and pest control practices (Johansson et al., 2002; Uri, 1998; Yiridoe et al., 2005). Further, global climate change, and its potential influence on water availability and the distribution and emergence of new pests and pathogens (Boland et al., 2004; Johansson et al., 2002), will add to the pressures on irrigation water resources. It is clear that nursery and greenhouse managers require new technologies and management strategies that will empower them to meet these resource and pest challenges as well as the environmental, social and legislative shifts facing the industry (Hong and Moorman, 2005). Adaptation to emerging market, social and environmental realities will rely upon improvements in resource utilization efficiency, creation of value added products, and empowerment of growers to rapidly respond to dynamic consumer preferences. Effective water and pest management strategies that can deliver substantial savings in an environmentally benign fashion are an important component of future greenhouse and nursery management strategies. Aqueous ozone (O$_{3(aq)}$) technology can eliminate pathogens and many chemical contaminants in a wide range of water and wastewater streams, without leaving many of the harmful chemical residues associated with other treatment technologies, such as chlorination. These properties make the technology attractive to horticultural producers, however data regarding the phytotoxicity of aqueous ozone is lacking (Fujiwara and Fujii, 2002).

11 The material presented in this chapter is taken in whole or in part from a paper of the same title appearing in HortScience 44(3):774-780. (2009).
Ozone ($O_3$) is a tri-atomic allotrope of oxygen most commonly associated with interception of high energy UV radiation in the Earth’s stratosphere, or as a component of photochemical smog, a significant tropospheric pollution issue. Ozone gas has known and well-characterized phytotoxic effects, such as reduced photosynthetic capacity, foliar reddening, and necrosis (Davison and Barnes, 1998; Fiscus et al., 2005; Fuhrer and Booker, 2003; Heath, 1996; Sandermann Jr., 1996). There is limited yet compelling evidence that the phytotoxic properties of ozone are less when the ozone exposure is in an aqueous form (Fujiwara and Fugii, 2002; McDonald, 2007; Sloan and Engelke, 2005). There is additional evidence that low-level ozone exposure can stimulate oxidative stress adaptation without visible evidence of damage (Chamnongpol et al. 1998; Kovalchuck et al. 2003; Pell et al., 1997; Ranieri et al., 1996; Reiling and Davison, 1995; Zheng et al., 2002). Further to this effect, low dose ozone has also been implicated in the triggering of systemic acquired resistance (SAR) responses that convey plant resistance to further pathogen attack (Durrant and Dong, 2004; Pell et al., 1997; Rao and Davis, 2001). The vast amount of research that has characterized the phytotoxicity of gaseous ozone may have inadvertently lead to an oversight of the prophylactic use of ozone, in the aqueous form, to address common nursery and greenhouse production issues.

Aqueous ozone has long been used as a water treatment technology in a diverse range of applications, including limited use in the treatment of greenhouse irrigation water (Ehret et al., 2001; Guzel-Seydim et al., 2004; Ingura et al., 2004; Rice, 1997; Runia, 1994, 1995). A strong oxidation potential (e.g. 2.07eV) coupled with a short persistence period (e.g. seconds to tens of minutes) has made aqueous ozone an ideal microbial and chemical contaminant control agent in many commercial settings (e.g. municipal water treatment, food processing, sewage treatment, post-harvest storage). These same properties also lend themselves to applications in greenhouse and nursery environments. Particular interest lies in the potential of aqueous ozone as an irrigation water remediation technology and as a means to control pathogens without leaving a chemical residue on the consumer product; a drawback of many current pest control strategies, and a growing concern amongst consumers (Miles and Frewer, 2001; Woese et al, 1997).

When introduced into water, the half-life of ozone is variable, but typically it is very short as the ozone rapidly reacts with microorganisms and oxidation-prone organic compounds in the water (Beltrán, 2004). Ozone that is not consumed through these pathways quickly converts to reactive oxygen-containing free radical species, all having very short half-lives, and eventually to diatomic oxygen ($O_2$) (Beltrán, 2004; von Guten, 2003). The result of this reversion is the absence of direct chemical residues associated with the treatment. This is not to say that other secondary disinfection by-products (DBP) are
absent, although the general consensus is that the DBPs formed under ozonation are less problematic than those formed by other common water treatment technologies, such as chlorination (Rakness, 2005). The act of generating and dissolving ozone in irrigation water also leads to enhanced dissolved oxygen content. Enhanced oxygenation has been shown to have benefits in terms of improved productivity and pathogen control in greenhouse production (Zheng et al., 2007).

The objectives of this study were to: 1) determine the loss of ozone from solution during overhead irrigation; 2) quantify foliar damage under overhead irrigation with aqueous ozone; and 3) characterize the impact of aqueous ozone on plant productivity.

MATERIALS AND METHODS

PLANT MATERIAL

Five economically-significant woody perennial nursery species [Salix integra 'Hakuro Nishiki' Thunb.; Weigela florida 'Alexandra' Thunb.; Spiraea japonica 'Goldmound' L.f.; Hydrangea paniculata 'Grandiflora' Siebold; Physocarpus opulifolius 'Summer Wine' L. Maxim.] were selected for the six-week experiment. The plant material for this study was provided by Canadale Nurseries Limited (St. Thomas, Ontario, Canada; 42°47′58″N 81°12′52″W). A detailed description of the plants used in this study and growth media employed are described in Cayanan et al. (2008).

The plants were moved from the field into a greenhouse at the University of Guelph (Guelph, Ont., Canada; 43°33′N 80°15′W). The move was made in August 2006 to prevent the onset of dormancy and provide more uniform growth conditions. The plants were allowed to acclimate for five weeks prior to commencing the six-week treatment regime in October 2006. The greenhouse environment control system (Argus Control Systems Ltd., White Rock, British Columbia, Canada) was set at 25°C/18°C day/night with a 16-h photoperiod, supplemented by artificial lighting consisting of 400 and 600 W high pressure sodium and metal halide lights, respectively.

AQUEOUS OZONE PREPARATION AND APPLICATION

Aqueous ozone solutions (control; 0.5 mg·L⁻¹; 1.5 mg·L⁻¹; 3.0 mg·L⁻¹; 6.0 mg·L⁻¹ aqueous ozone) were prepared at the time of irrigation. Deionized water was fed into a mass transfer loop (Shaw Mixer™, Purification Research Technologies Incorporated, Guelph, Ont., Canada) running at loop pressure of 206.8 kPa. The mass transfer loop impinged an ozone gas stream, via a venturi injector (Mazzei 584, Bakersfield, Calif.), on the irrigation water stream. The design of the mass transfer loop was such that the water would undergo the impingement process approximately five times before exiting
the loop system. The ozone gas used in the mass transfer loop was generated by passing ambient air through an oxygen concentrator (Workhorse-8 5665, SeQual Technologies Inc., San Diego.) that removed the majority of the nitrogen from the air stream, passing the concentrated oxygen (90-95% O₂) through to a corona discharge ozone generator (Model 1500P, Clearwater Tech LLC. San Luis Obispo, Calif.).

A side stream of the solution containing dissolved ozone was drawn off the mass transfer loop and directed to a dissolved ozone monitor (Model W1, INUSA Inc., Norwood, Mass.). The remaining bulk solution was diverted to a bypass until the appropriate dissolved ozone level was achieved, at which time the solution was redirected to the irrigation line supplying the plants at the respective treatment levels. The feed lines (one line for each treatment) were configured in a loop that passed through all the blocks. Branch lines from each of the treatment lines fed overhead emitters at each of the main plots in each block (five branches per treatment line). Each daily treatment application was maintained for 7.5 minutes, which allowed for an average water delivery of 1 L·plant⁻¹.

To minimize the effects of extraneous ozone off-gas (between main plots) during treatment, and to prevent overspray from affecting neighbouring plots, an open top enclosure was placed around each main plot during treatment. Ambient atmospheric ozone levels were also monitored (Model 1004H, Dasibi Environmental Corporation, Glendale, Calif.) and two large carbon filters, with a total maximum air handling capacity of 48 m³·min⁻¹, were placed at random locations throughout the growth area during each daily treatment, to prevent gas phase (ozone) cross-contamination amongst treatments when the high ozone doses were being applied. The system provided sufficient control over extraneous off gassing such that the ambient ozone levels only periodically and transiently exceeded historic (1980-2006) ambient one-hour maximum ozone levels for the province of Ontario (air quality standard for ozone 1-h maximum is 80 ppb) (Ontario Ministry of the Environment, 2007).

**Aqueous Ozone Determination at Canopy Height**

It was not feasible to control aqueous ozone levels at each emitter for each treatment level. Ozone levels were set and maintained by drawing off a sample prior to sending the solution to the distribution system. The length of the distribution system was minimized to reduce the amount of ozone lost during transport to the plants; however, ozone is lost from solution when that solution is atomized or depressurized, as is the case at the discharge of the emitters. This known loss potential necessitated the measurement of residual ozone at the emitter discharge and at an average canopy height. The canopy height was set to 0.3 m below the emitter head, at a distance of 0.3 m from the base of the emitter tower.
These distances translate into an approximate spray travel distance of 0.47 m through the air, calculated as ¼ the circumference of a circle with a radius of 0.3 m. Using the gravimetric indigo colorimetric method (Yates and Stenstrom, 2000), residual ozone concentrations were evaluated in triplicate at each emitter and at each treatment level.

**GROWTH EVALUATION**

Upon completion of the six-week treatment period, all plants were destructively sampled. Leaves were excised and passed through a leaf area meter (Model LI-3100C, LiCor, Lincoln, Nebraska USA) and then placed in drying bags along with the woody components of the stem. The combined stem fresh weight was measured (Sartorius LC12000P, Goettingen, Germany) with the average mass of the bag removed. Dry weight was determined by placing the bags in a drying oven at 70°C until a constant dry weight was obtained, at which point the dry weight was recorded. The leaves from a randomly selected plant from each species in each block and each treatment level were separated for leaf damage index determination, after which they were also dried to a constant weight. The roots of all the plants were washed by a combination of soaking, agitation, and rinsing in a stainless steel sink. The washed roots were placed in paper drying bags, and dried to a constant weight. Given the time requirements of the root washing process, the pots were stored in the dark at 4°C until they could be processed. In the case of *W. florida*, which flowered during the final 10 d of the study, the total number of flowers was recorded.

**LEAF DAMAGE INDEX**

Visible leaf damage is a key indicator of plant performance in nursery crops, and the potential for visible injury due to aqueous ozone application could have a serious impact on grower and consumer acceptance. To quantify the damage in a more objective fashion, an image analysis protocol was developed to determine the percent damage occurring in a randomly selected subset of the total canopy. Leaves from a randomly selected plant from each species in each treatment were positioned on a white, laminated card (22 cm x 30 cm) coated with a mutiuise mild adhesive (Scotch™ glue stick) that allowed the leaves to remain in place during scanning but also allowed for easy removal from the card. The card was labelled with an identification code and placed on a flatbed scanner (A920, Dell, Round Rock, Texas). The card was scanned at a resolution of 300 dots per inch and saved to hard disk as a bitmap image. The images were then pre-processed to remove any image components that were not leaf material (label and scale reference), and to crop the images to a uniform size appropriate for each species (Automator Version 2.0.1 (156), Apple Inc., Cupertino, CA.). Each image was then digitally enhanced
to differentiate the damaged and healthy regions (Fig. 5.1A and B) of the leaves in each image (GIMP 2.4.3 for Mac OS X, GNU Image Manipulation Program, http://www.gimp.org). The specific image modifications were different for each of the five species examined, as the colour of healthy and damaged tissue varied among the species. Threshold, saturation and hue manipulation allowed for the greatest damage differentiation (Fig. 5.1C). Common to all the images was the removal of the background color and selection of the damaged and healthy regions by pixel color, which varied amongst species. The removal of the background was accomplished by adding a transparent alpha channel to the image and coloring the dominant background color (i.e. white) to that channel, effectively removing the background from the range of analysis colors. After modification of the images (Fig. 5.1B and C), the damaged regions were easily distinguishable from the healthy regions. The healthy regions were then selected by similar colour and isolated from the damaged regions (Fig. 5.1D). Using the histogram dialogue function, the number of pixels selected as healthy tissue was recorded. Inverting the selection (Fig. 5.1E) allowed for the total number of pixels associated with damaged tissue to be determined. The Leaf Damage Index (LDI) was calculated by dividing the damaged tissue pixel count by the total leaf area pixel count. The LDI accounts for all damaged tissue, regardless of origin, so the differences evaluated are relative to the control, which was considered to be the baseline.

**Leaf Gas Exchange**

Leaf intercellular CO₂ concentration, stomatal conductance, and net CO₂ assimilation rate were measured on the last fully expanded leaf at week two and week four of the experiment. Measurements were made using a portable photosynthesis measurement system (Model LI-6400; LiCor Biosciences, Lincoln, NE).

**Flower Production**

Of the species examined, only *W. florida* reached flowering stage. Flower numbers were recorded at weekly intervals and the total number of flowers produced per plant was calculated over the six-week treatment period.
Fig. 5.1: Representative steps in the image analysis used to quantify leaf injury (e.g. Leaf Damage Index). The representative species shown is *S. integra*. The original, unmodified image (A) illustrates the range of injury symptoms. The leaf to the left represents the most severe injury; the middle leaf represents moderate damage; the right side leaf represents minor/no damage. (B) Contrast and brightness enhancements aid in differentiating damaged sections. (C) Hue and saturation levels were optimized to split the damaged and healthy tissue into two narrow color bands that are easily distinguished. (D) Healthy tissue pixels were selected by color using the ‘select by color’ tool in the image manipulation software (GIMP). The selected tissue was then temporarily deleted and the pixel count of the damaged tissue was read. (E) The selection process was reversed with the damaged tissue pixels being removed. This was done to confirm the process and to ensure that the total pixel count was accurate. The images presented are only intended as a sample. The actual analysis was carried out on a much larger scale to ensure that the individual LDI was representative of the entire plant. The black background has been added for publication contrast.
EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The experiment was arranged as a randomized complete block design (RCBD), with five blocks containing five treatment levels. Ozone treatment levels were randomly assigned to one of five emitters in each block. Three plants from each species were arranged in a cluster and placed around the emitter.

Statistical analysis was performed using the generalized linear model in SAS 9.13 (SAS, Cary, North Carolina). Treatment means were separated with the least significant difference test if the main treatment effect was significant at $P \leq 0.05$. Leaf Damage Indices were analysed through a one-way ANOVA, followed by Dunnett’s post-test using GraphPad Prism version 5.0a for Mac (GraphPad Software, San Diego, CA).

RESULTS

APPLIED OZONE DOSE

Dissolved ozone concentrations at an average plant canopy height 0.3 m below the emitters were approximately 30% that of the concentrations measured at the emitter discharge (Fig. 5.2). The actual amount of ozone remaining in the solutions after travelling 0.47 m through the air was 0.17, 0.45, 0.85 and 1.39 mg·L$^{-1}$ for the respective non-zero ozone treatments (Fig. 5.2).
Fig. 5.2: Ozone loss from solution as a result of spraying. The value (%) above each group is the calculated percent ozone loss from solution after the solution is discharged from the emitter and travels an average distance of 0.47 m through the air. Error bars are ± SE based on 30 measurements. Inset: Gravimetric indigo method sampling of aqueous ozone at canopy height (plants removed).
**Final Harvest Data**

In all five species, the growth patterns and leaf damage index impacts were similar (Fig. 5.3 A-F; Table 5.1). *Salix integra* was the most vigorous species in terms of new growth; the results for this species are presented graphically (Fig. 5.3 A-F) and are considered representative of the overall trend for all five species (Table 5.1). There was a clear damage threshold between 1.5 and 3.0 mg·L⁻¹; however, the specific threshold value could not be refined any further given the scope of this study. In the two highest treatments it was observed that the new growth initiated after the third week of the study showed greatly reduced leaf damage, especially in *S. integra*.

**Leaf Gas Exchange**

The net CO₂ assimilation rate (A), intercellular CO₂ concentration (cᵢ) and leaf stomatal conductance (gₛ) data are presented in Fig. 5.4, Table 5.2 and Table 5.3. All four species measured at week two (Fig. 5.4; Table 5.2), showed a reduced (or variable) CO₂ assimilation rate, intercellular CO₂ concentration and leaf stomatal conductance, relative to the control. The variation in the response between treatments was not maintained when measured at week five (Fig. 5.4; Table 5.3).

**Flower Production**

Overall, there was a minor increase in flower number at the 0.5 mg·L⁻¹ treatment level and a decrease in the number of flowers produced at 6.0 mg·L⁻¹ in *W. florida*, the only plant that flowered during this study (Fig. 5.5).
Fig. 5.3: Response of *S. integra* to overhead irrigation with different aqueous ozone solutions. A) Leaf Area; B) Leaf Damage Index (LDI); C) Shoot Dry Weight (SDW); D) Root Dry Weight (RDW); E) Shoot Fresh Weight (SFW); F) Shoot Height. Bars represent the average for each parameter ± SE at each dose level measured during the destructive sampling of the plants after six weeks of daily treatments. Treatment means with the same letter are not different at $P \leq 0.05$. 
Table 5.1: Treatment means for the six harvest metrics measured for *Spiraea japonica*, *Weigela florida*, *Hydrangea paniculata* and *Physocarpus opulifolius*.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>OZONE (mg·L⁻¹)</th>
<th>LEAF AREA (cm²)</th>
<th>LEAF DAMAGE INDEX</th>
<th>SHOOT DRY WEIGHT (g)</th>
<th>ROOT DRY WEIGHT (g)</th>
<th>SHOOT FRESH WEIGHT (g)</th>
<th>SHOOT HEIGHT (cm)</th>
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</thead>
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<td></td>
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<td>194 a&lt;sup&gt;z&lt;/sup&gt;</td>
<td>0.193 a</td>
<td>4.35 a</td>
<td>2.10 a</td>
<td>8.94 ab</td>
<td>26.40 a</td>
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<tr>
<td></td>
<td>0.5</td>
<td>232 ab</td>
<td>0.250 a</td>
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<td>2.14 a</td>
<td>9.37 ab</td>
<td>28.13 a</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>286 abc</td>
<td>0.249 a</td>
<td>4.59 a</td>
<td>1.95 a</td>
<td>9.87 a</td>
<td>28.53 a</td>
</tr>
<tr>
<td></td>
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<td>154 bc</td>
<td>0.497 b</td>
<td>4.65 a</td>
<td>2.43 a</td>
<td>9.24 ab</td>
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<tr>
<td></td>
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<tr>
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<td><em>W. florida</em></td>
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<td>28.47 bc</td>
</tr>
</tbody>
</table>

<sup>z</sup> Treatment means (within columns and species) with the same letter are not different at $P≤0.05$
Fig. 5.4: Photosynthesis and gas exchange summary. A) Leaf net CO₂ assimilation rate; B) intercellular CO₂ concentration; C) stomatal conductance for *S. integra* at five aqueous ozone treatment levels, measured at weeks two and five. Columns within each group with the same letter are not significantly different at $P \leq 0.05$. 
A

Net CO₂ Assimilation Rate
(µmol·m⁻²·s⁻¹)

Week 2 Week 5

Control
0.5 mg L⁻¹
1.5 mg L⁻¹
3.0 mg L⁻¹
6.0 mg L⁻¹

B

Internal CO₂ concentration
(µmol·mol⁻¹)

Week 2 Week 5

C

Stomatal Conductance
(mmol·m⁻²·s⁻¹)

Week 2 Week 5
Table 5.2: Leaf net CO\(_2\) assimilation rate (A), intercellular CO\(_2\) concentration (c\(_i\)), and stomatal conductance (g\(_s\)) for *W. florida*, *H. paniculata*, and *P. opulifolius* at five treatment levels (week 2)

<table>
<thead>
<tr>
<th>SPECIES(^2)</th>
<th>[O(<em>3)]</em>{aq} \text{ mgl·L}^{-1}</th>
<th>A \text{ µmol·m}^{-2}·s^{-1}</th>
<th>c(_i) \text{ µmol·mol}^{-1}</th>
<th>g(_s) \text{ mmol·m}^{-2}·s^{-1}</th>
</tr>
</thead>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>304 ab</td>
</tr>
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<td>8.61 a</td>
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<td>339 ab</td>
</tr>
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<td>362 a</td>
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<td></td>
<td>6.0</td>
<td>7.45 ab</td>
<td>344 ab</td>
<td>314 ab</td>
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<td><em>H. paniculata</em></td>
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<td></td>
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<td><em>P. opulifolius</em></td>
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<td>1.5</td>
<td>11.27 a</td>
<td>345 a</td>
<td>514 a</td>
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<td></td>
<td>6.0</td>
<td>7.91 c</td>
<td>361 c</td>
<td>541 a</td>
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</table>

\(^{2}\) Treatment means (within column) with the same letter do not differ significantly at P≤0.05.

\(^{2}\) *S. japonica* is excluded as its leaves were too small for the measurement chamber; *S. integra* is presented in Fig. 5.4.
Table 5.3: Leaf net CO$_2$ assimilation rate (A), intercellular CO$_2$ concentration ($c_i$), and stomatal conductance ($g_s$) for *W. florida*, *H. paniculata*, and *P. opulifolius* at five treatment levels (week 5)

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>$[O_3]_{aq}$ (mg·L$^{-1}$)</th>
<th>A (µmol·m$^{-2}$·s$^{-1}$)</th>
<th>$c_i$ (µmol·mol$^{-1}$)</th>
<th>$g_s$ (mmol·m$^{-2}$·s$^{-1}$)</th>
</tr>
</thead>
<tbody>
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<td><em>W. florida</em></td>
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<td></td>
<td></td>
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</tr>
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<td>206 a</td>
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<td>235 a</td>
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<td><em>H. paniculata</em></td>
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</table>

z Treatment means (within column) with the same letter do not differ significantly at $P \leq 0.05$

y *S. japonica* is excluded as its leaves were too small for the measurement chamber; *S. integra* is presented in Fig. 5.4.
Fig. 5.5: Average number of flowers per plant for *W. florida* after six weeks of irrigation with aqueous ozone solutions [1 L per plant per day]. Columns with the same letter do not differ at $P \leq 0.05$. Data are mean ± SE.
**DISCUSSION**

Results indicated that there was a reasonably defined aqueous ozone phytotoxic threshold under the exposure conditions employed, for the five species examined. Given these data, and taking into account experiment limitations, it can be stated that this threshold lies between 1.5 and 3.0 mg·L\(^{-1}\), at the emitter discharge, over a 7.5 min application period. Below 3.0 mg·L\(^{-1}\) there were no negative effects of the ozone application compared to the control. Furthermore, the gas exchange data (Fig. 5.4; Table 5.2; Table 5.3) suggested that there was an adaptation to the oxidative environment imposed. This trend was supported by visual inspection of foliage (e.g. *S. integra*) produced during the treatment period. The in-treatment foliage showed reduced incidence of damage compared to foliage present before the treatments commenced. This reduced damage was seen in the leaf image analysis data (Fig. 5.3; Table 5.1) in which, even at the highest treatment levels, the percent damage was still below 50%. Leaves produced before the treatments (3.0, 6.0 mg·L\(^{-1}\)) commenced were severely damaged (see Fig. 5.1 severe damage example), with an estimated damage index of 0.9. Foliage produced after treatments commenced had minor to moderate damage. The aggregate leaf damage served to reduce the overall LDI for the highest treatments, although significant damage was still realized overall at these treatment levels.

Aqueous ozone loss as a result of spraying proved to be substantial (Fig. 5.2). This loss confounded the conclusions to some degree, as the concentration realized at the leaf surface was considerably lower than the concentration present in the distribution system and at the emitter discharge. Given this loss, the threshold, as measured at the average canopy height, can be estimated as 30% of the measured threshold in the distribution system. When developing protocols for irrigation system maintenance using aqueous ozone, this loss along with the corresponding increase in gas phase ozone must be a design consideration.

In this study, ozone off-gas was contained to a greater degree than it would be in an open-air nursery situation or during summer greenhouse production when vents would be fully opened. Even with the greater degree of closure, atmospheric dilution and the short half-life of ozone resulted in only minor gas phase ozone increases. The ambient ozone concentrations reached in the greenhouse were not atypical for a normal summer day in eastern North America (*Ontario Ministry of the Environment, 2007*). Although ozone gas levels did rise in the greenhouse the increases were transient, resulting in only minor periodic exposures, compared to typical smog events, well below the levels that are associated with long-term production influences (*Langebartels et al., 1998*). These transient elevated levels may be a concern from a worker safety point of view and must be considered in any future system development.
The factors affecting plant response to ozone are numerous and diverse. Previous exposure, water status, stomatal functioning, genetics, duration and severity of the exposure, cuticular composition, environmental conditions (e.g. wind speed, humidity, temperature) and plant developmental stage are just a few of the parameters governing the mass transfer of ozone into a plant and the physiological responses occurring thereafter (Heagle, 1989; Kerstiens and Lendzian, 1989; Lyons and Barnes, 1998; Pasqualini et al., 2002; Sandermann Jr., 1996). For these reasons a simple, universal toxicity threshold cannot be determined, yet the evidence presented clearly indicates that the crop species examined can tolerate aqueous ozone concentrations that are useful in terms of pathogen and chemical contaminant control in nursery and greenhouse irrigation systems.

The phytotoxic effects of gaseous ozone are reasonably well understood, although many questions still remain regarding stomata-controlled mass transfer, internal distribution of ozone, direct versus indirect effects, and physiological systems affected within the leaf and plant proper (Altimir et al., 2008; Fuhrer and Booker, 2003; Langebartels, 2002; Pell et al., 1997; Sandermann Jr., 1996). Application of ozone in the aqueous state further complicates the situation by influencing mass transfer dynamics, thermodynamic conditions, reaction pathways, and boundary layer conditions. In addition, the method of application may also play a significant role in determining the phytotoxic threshold, as this could influence ozone mass transfer from the irrigation solution to the canopy atmosphere (Fujiwara and Fujii, 2004). The results obtained under the presented experimental conditions support the work of others (Fujiwara and Fujii, 2002) that suggested that ozone applied in aqueous solution does not interact with plants in the same fashion as gas phase ozone does. The mechanisms of this difference remain to be determined, and will no doubt hold strict license on the design and application of aqueous ozone systems in a nursery setting.

The aqueous ozone phytotoxic threshold established under the present experimental conditions was sufficiently high to accommodate the practical application of aqueous ozone in irrigation distribution systems. The application of ozone at these levels could allow for the control of algae and biofilm development, and has potential to reduce pathogen populations, a particularly important consideration when recirculating irrigation systems are employed. In the absence of any direct plant productivity benefits, aqueous ozone could still improve profit margins by reducing irrigation system maintenance and reducing the pathogen load associated with untreated and recycled irrigation water.

Given that ozone exposure, as a component of photochemical smog, is a significant plant production issue in many key North American and European nursery production areas (Heagle, 1989), it is intriguing
to conceptualize ozone ‘immunization’ protocols that enhance natural tolerance to the oxidant. Methods of artificially inducing ozone resistance exist (Heath, 1996; Lenhardt, 1993; Heagle, 1989); however, it may be that the best option for stimulating tolerance development is through the application of ozone itself. The tight control that modern systems offer for the delivery and dose of aqueous ozone, and the adaptation to ozone that has been demonstrated by others (Kovalchuck et al., 2003; Lyons and Barnes, 1998), it stands to reason that it would be possible to slowly acclimate seedlings and young nursery stock to ozone stress by applying it early in the growing season when pollution events are rare. Early adaptation may help mitigate the visible damage that can result after a substantial smog event, damage that can dramatically reduce the value of the nursery product.

CONCLUSIONS

Under the conditions examined, aqueous ozone can be applied to select perennial woody nursery species in overhead irrigation systems at concentrations < 1.5 mg·L⁻¹, as measured at the point of discharge. Although the scalability of these results is debatable, the data does provide the baseline for expanded field trials.

Overhead irrigation systems, unlike the drip irrigation systems previously examined, have a greater loss of ozone to the atmosphere surrounding the emitters. Ozone gas above critical thresholds is known to be phytotoxic and care must be taken when attempting to utilize it as an irrigation management tool, but this should not negate the exploration of the application. A great deal more work is required to develop solid recommendations for growers in terms of employing aqueous ozone in their overhead irrigated production systems; however, the potential benefit, as demonstrated herein, is worth the research investment.
CHAPTER 6

LIVERWORT CONTROL: AN ANCILLARY ROLE FOR OZONE-BASED IRRIGATION WATER TREATMENT SYSTEMS?  \(^{12}\)

INTRODUCTION

*Marchantia polymorpha* L., a common thalloid liverwort\(^{13}\) (Fig. 6.1 A-C), is a significant weed species in nursery and greenhouse operations across North America and Europe, being particularly problematic in propagation houses where the environmental conditions maintained for newly established potted plants are ideal for rapid liverwort establishment (Svenson et al., 1997). Liverwort reproduces sexually through spore formation (Fig. 6.1 B), and asexually via tissue fragmentation and the production of gemmae; clonal fragments produced in specialized structures called gemma cups (Fig. 6.1 C) (Altland et al., 2003; Svenson et al., 1997). Combined, these reproductive strategies enable the rapid distribution and development of liverwort on the surface of nursery container growth substrates (Fig. 6.1 A).

In potted plant production, liverwort infestations present a clear impediment to water and nutrient infiltration (Fig. 6.1 A), thereby reducing the growth and value of the crop (Svenson et al., 1997). This diversion results in higher water and fertilizer demands, which translates to greater production costs, reduced productivity, and environmental impacts in the form of excessive water taking and increased nutrient discharge from the production facility.

A heavy liverwort infestation also provides habitat for other pests and pathogens, such as, fungus gnats (*Bradyisia* spp.; *Sciaridae*), snails (e.g. *Helix* spp.), slugs (e.g. *Deroceras* spp.), and a host of microbial threats such as *Fusarium* spp., and *Pythium* spp. (Svenson et al., 1997). Additional costs to control these pests, combined with production losses resulting from their activity, further erode profit margins.

\(^{12}\) The material presented in this chapter is taken in whole or in part from a paper of the same title appearing in *HortScience* 47(3):361-367. (2012).

\(^{13}\) for the purposes of this paper the term liverwort will refer only to the species *M. polymorpha*
Fig. 6.1: Liverwort in nursery production. A) An example of severe liverwort infestation in container nursery stock. Liverwort mats on the substrate surface interfere with water and nutrient penetration while providing habitat for pests and disease vectors. B) Female (archegoniophore) and male (antheridiophore) reproductive structures. C) Gemma cups.
The impacts, on profit margins, of a significant liverwort infestation continue to be realized once a potted crop reaches marketable size. The presence of liverwort is considered unsightly and often taken as an indication of reduced quality or plant vigor, all of which impact the final valuation of the crop.

A significant amount of research has been conducted to evaluate chemical compounds for the control of liverwort (Newby, 2006). Svenson et al. (1997) provide a list of compounds purported to have some efficacy in the control of liverwort. Although potentially effective under prescribed conditions, many of the listed chemicals are not registered for liverwort control in many North American production regions. Lack of registered control products leaves growers with few options beyond hand removal.

Hand removal is a costly method of weed control by any measure and can increase the unit cost of production dramatically. Estimates put the cost of supplemental hand weeding (not exclusive to liverwort) at $1,235-$9,880 per ha (Case et al., 2005; Judge et al., 2004). In addition to the direct labor costs associated with hand removal, the physical removal of weeds also removes a portion of the upper layer of substrate (including surface-applied, slow-release fertilizer), thereby damaging roots in the upper segment of the pot. The cost of hand removal and the impacts that the practice has on substrate structure and root vigor necessitates continued effort to develop alternative control strategies.

Ozonation, the dissolution of ozone (O₃) gas in irrigation solutions, is an emerging agricultural water remediation technology that has garnered favor on both environmental and operational efficacy grounds. Ozone is a highly effective antimicrobial agent while also being reactive with many chemical contaminants (Beltrán, 2004) that may be present in irrigation source water. Furthermore, in a time when organic markets are outpacing traditional agricultural commodity markets, with organic products commanding significant price premiums (Kendrick, 2008), ozone is one of the few disinfection options compatible with organic production methods and certification bodies. Ozone's acceptance as an organically compatible intervention technology is based largely on the fact that there are no ozone residues remaining on the crop after application. Residual ozone, that which is not consumed as a part of the treatment, spontaneously reverts to diatomic oxygen (O₂) in a complex process that further enhances the antimicrobial effect.
This study has focused on aqueous ozone (O$_{3(aq)}$) as a potential component of an overall liverwort management program when the technology is already employed as an irrigation water remediation tool. Aqueous ozone has a long history of water and wastewater treatment applications and in recent years has also gained some momentum as an irrigation water treatment technology in nursery and greenhouse production (Ehret et al., 2001; Graham et al., 2009; McDonald, 2007). Operators that use ozonation as a component of their irrigation water treatment system tend to use it in batch format. The water is treated with ozone and stored in tanks to allow the residual ozone to revert to O$_2$. Alternatively, the solutions are passed through filters that breakdown the residual ozone.

The removal of ozone prior to distribution to the crop provides an opportunity for re-inoculation of the solution from biofilms found on the distribution system hardware (Stewart-Wade, 2011). The removal of the disinfecting agent also disallows any potential for in situ pathogen control through direct ozone contact with pathogens on the plant or growth substrate surfaces.

Justifiable prudence prompts the removal of ozone from irrigation solutions as ozone [gas] phytotoxicity is well established (Ashmore, 2005). Tropospheric ozone enrichment [photochemical smog] elicits phytotoxic reactions in a wide array of plant species over a range of concentrations (Bell and Treshow, 2002). Although phytotoxic, recent studies suggest that under conditions of controlled application in aqueous solution, ozone can be safely applied [foliar and directly to substrate] to select horticultural crop species (Fujiwara and Fujii, 2002; Graham et al., 2009; Graham et al., 2011b; Ohashi-Kaneko et al., 2009; Sloan and Engelke, 2005). There is also limited evidence that ozone application to the root zone can improve some plant performance metrics (Sloan and Engelke, 2005). The capacity to safely retain residual ozone in the irrigation solution during distribution to the crop is significant in that it may allow for the control of pests/pathogens throughout the irrigation system and may in fact have some efficacy in the control of pests at the plant/pot level (Fujiwara and Fujii, 2002).

Marchantia polymorpha, and thalloid liverworts in general, are distinctive within the plant kingdom in that they do not possess the guard cells required to actively regulate gas exchange between the bulk atmosphere and the thallus interior (Green and Snelgar, 1982). In place of a functional stomatal complex, liverwort has a pore structure that is a largely unregulated diffusion pathway. It is reasonable to surmise that this restricted capacity to regulate gas exchange would result in a greater flux of pollutant gasses relative to plants fully capable of regulating gas exchange. If this is the case, then liverwort should
exhibit a greater negative growth response to an application of ozone with all else being equal (i.e. no species specific antioxidant systems). It is on this premise that these studies were based.

The objectives of the presented studies were to: 1) examine contact time as a process control parameter for liverwort management; 2) establish initial aqueous ozone toxicity thresholds for liverwort; and 3) evaluate the response of liverwort to aqueous ozone at exposure levels and application frequencies that are consistent with the tolerance thresholds of select woody perennial nursery species established previously (Graham et al., 2009).

MATERIALS AND METHODS

PLANT MATERIAL PROPAGATION

Liverwort samples were collected from a nursery operation in southern Ontario (St. Thomas, Ont., Canada; long. 42°47'58" N, lat. 81°12'52" W) and brought to the University of Guelph (Guelph, Ont., Canada; 43°33'N 80°15'W) for propagation. The samples were separated into sections approximately 5 cm² and evenly (5 cm spacing) placed onto mineral wool propagation sheets (Grodan Inc., Milton, Ontario, Canada). The transplants were then placed on a propagation misting bed and kept between 18-22 °C under 400 W high-pressure sodium lights that delivered 90-100 umol·m⁻²·s⁻¹ at the tissue surface. The tissue was misted with DI water until wet, every 30 min during the 14-h photoperiod. Misting frequency was reduced to once every hour during the 10 h dark period. The transplants were fertilized with a modified Voth #5 solution (Voth, 1943) twice daily by misting with a hand sprayer until the surface of the tissue was wet. After a 5-7 day establishment period, during which the tissue anchored to media by newly formed rhizoids, the regular fertilizer regime was supplemented with a 5 mM Ca(NO₃)₂ solution three times per week.

EXPERIMENTAL UNIT PREPARATION

Apical sections of liverwort approximately 1 cm² were excised from the propagated parent material. Sets of four segments were selected, photographed against a white background with a scale and experimental unit ID clearly visible, then arranged radially on a 7 cm x 7 cm x 2 cm block of mineral wool (Gro-Slab Expert 20/77 A2W, Grodan Inc., Milton, Ontario, Canada). The block was pre-soaked in deionized water for 1 h followed by a soaking in a 1:10 dilution of a modified Voth #5 solution. The
populated blocks were then placed on a misting bed for 14 d (Expt. 1 and 2) or 7 d (Expt. 3), prior to treatment initiation, during which rhizoid formation secured the individual segments to the mineral wool blocks. The blocks were misted with a full strength modified Voth #5 solution twice daily during the establishment period, and once daily during the treatment period.

**AQUEOUS OZONE PREPARATION**

Aqueous ozone is unstable and as such, solutions were prepared at the time of treatment application. Tap water (17-21 °C) was fed into a mass transfer loop (Shaw Mixer™, Purification Research Technologies Incorporated, Guelph, Ont., Canada) running at loop pressure of 206.8 kPa. The system impinged ozone gas, via a venturi injector (Mazzei 584, Bakersfield, Calif.), on the irrigation water stream. The ozone gas used in the mass transfer loop was generated by passing ambient air through an oxygen concentrator (Workhorse-8 5665, SeQual Technologies Inc., San Diego.) that removed the majority of the nitrogen from the air stream, passing the concentrated oxygen (90-95% O$_2$) through to a corona discharge ozone generator (Model 1500P, Clearwater Tech LLC. San Luis Obispo, Calif.). Excess ozone [not dissolved] in the system flowed to the top of the loop where it was discharged to an off-gas destruct unit (Model OCD11, Clearwater Tech LLC. San Luis Obispo, Calif.).

**AQUEOUS OZONE APPLICATION**

To achieve stable ozone exposure regimes, a dunking system was constructed in which the experimental units could be submerged for the prescribed time period. The assembly consisted of a bottom-fed reservoir connected to the output of the mass transfer system. In the first two trials, the system was set up as an overflow reservoir that would continuously deliver the ozone-containing solution to the bottom of a 2.5 L reservoir, where it would flow up past the experimental units and out over a spillway. In this system the ozone generator was set to fixed outputs that provided relatively stable ozone concentrations in the treatment stream. In the third trial, the system was expanded and automatic feedback control was added. The reservoir was enlarged to 50 L and a mixing port was incorporated into the bottom feed. The mixing port consisted of a 15 cm length of 2.5 cm diameter PVC pipe with 0.8 mm holes drilled in a radial pattern along the length of the pipe. This allowed for good mixing of the input solution within the reservoir. A secondary stream of the mixed solution was taken off the reservoir and directed to a dissolved ozone monitor (Model Q45H, Analytical Technologies Inc., Collegeville, PA.). The monitor was equipped with an output feedback control loop that was tuned to maintain the bulk
solution at the ozone set point of 0.5 mg·L⁻¹. A submersion platform was also added to the system to allow all 14 experimental units to be treated at the same time.

Three separate experiments (Expt. 1, 2 & 3) were conducted. The first two experiments were conducted to determine if contact time was suitable as a control parameter when the objective is to control a plant pest rather than a microbial pathogen. Expt. 1 modulated the concentration at a fixed exposure time, while Expt. 2 held the ozone concentration constant while modulating the exposure time. Expt. 3 was designed to evaluate liverwort response to application frequency at CTs previously determined to be acceptable for woody perennial production (Graham et al., 2009).

**Expt. 1.** This experiment was set up as a completely randomized design (CRD) with six treatment levels and ten experimental units per treatment (n=10). Treatment allocation on the CRD grid was determined using IRRISTAT software (International Rice Research Institute, Los Baños, Philippines). Individual experimental units were assigned at random to each position on the grid using a random number series (www.Random.org). The treatments consisted of a 30 s exposure to solutions with [O₃(aq)] of 0.0 mg·L⁻¹, 0.5 mg·L⁻¹, 1.5 mg·L⁻¹, 2.5 mg·L⁻¹, 3.5 mg·L⁻¹, and 5.0 mg·L⁻¹ which translated to contact times of 0, 0.25, 0.75, 1.25, 1.75, and 2.50 mg·L⁻¹·min, respectively. The treatments were applied for five consecutive days, after which a two-day recovery period was provided before harvesting the tissue. Total thallus area (cm²) was measured with a leaf area meter (Model LI-3100C; LiCor, Lincoln, NE) and dry weight was measured with a standard analytical balance (Model TE124S; Sartorius AG, Goettingen, Germany) after the representative samples had reached a constant dry weight at 65 °C.

**Expt. 2.** This experiment was also set up as a CRD with six treatment levels and ten experimental units per treatment. The treatments consisted of exposures for 0, 30, 45, 60, 75 and 90-s to a 2.0 mg·L⁻¹ O₃(aq) solution, which translated into contact times of 0, 1.0, 1.5, 2.0, 2.5, and 3.0 mg·L⁻¹·min, respectively. The layout and experimental unit allocation were conducted as described in Expt. 1. The treatments were applied for five consecutive days, after which a two-day recovery period was provided. At this stage five units from each treatment were randomly selected for harvest (n=5). The remaining five experimental units were allowed to grow for an additional seven days before harvesting (n=5) to determine how well liverwort would recover from the treatments. Total thallus area and dry weight were measured as in Expt. 1 for each harvest period. Additionally, the chlorophyll content index (Model CCM-200; Opti-Sciences, Hudson, NH) was measured.
**Expt. 3.** A factorial treatment design (2 x 3; n=14) was utilized for this experiment, with O$_3$(aq) concentration and frequency of application as treatment factors. There were two levels of O$_3$(aq) concentration (0.0 mg·L$^{-1}$ and 0.5 mg·L$^{-1}$) and three frequency levels (one, three and five applications per week). All treatment applications were 7.5-min in duration and the treatments were maintained for 28-d. Treatment order on any given day was randomized first by O$_3$(aq) concentration then by frequency of application. During treatment application all experimental units were removed from the growth system and transported to the treatment facility to minimize variations arising from periodic exposure to different environmental regimes. In addition to the metrics already described for Expt. 1 and 2, the following parameters were also quantified.

**Gemma Cup Count:** The total gemma cups present at treatment initiation and treatment termination were counted. A 4x-magnifying lens was used during the count so as to include primordial cups (i.e. initiated but not fully developed) in the total count.

**Gemmae Counts, Average Size, Total Area:** After the final treatment application, all experimental units were thoroughly rinsed to remove as many gemmae as possible from their cups. The plants were then returned to the growth facility for four days to allow new gemmae to develop. After four days each block was inverted and placed on a filter disk (7.0 cm diameter Q5 quantitative disk; Fisher Scientific, Pittsburgh, PA) moistened with deionized water. Slight pressure was applied to the block to help release the gemmae and have them make contact with the filter disk, after which it was lifted and rotated a quarter turn and returned to the filter disk. The process was repeated until a complete rotation was made. The blotted filter disk was then placed in a petri dish and photographed against a black background with a scale present in the field of view. The disks were then wetted with 300 µL of a modified Voth #5 solution, covered and placed on the factorial grid in the same position that the mineral wool block was removed from. The digital images were analyzed (average size, total area, and total number) using the ‘analyze particles’ macro in ImageJ at a threshold set point of 64:125 (Rasband, 1997).

**Removal Force.** The strength and degree with which liverwort binds to the growth substrate influences how much media is removed and how much crop root disturbance could occur during hand removal. The maximum resistive force experienced during the removal of the tissue from the mineral wool block was measured with a force gauge (9812-FG-20KG, Control Company, Friendswood, TX.). The gauge was
connected to the thallus tissue through a fork inserted between the thallus tissue and the mineral wool. The tissue sample was secured to a stable surface and the force gauge was lifted, via a scissor-lift, until the tissue released from the mineral wool. The force gauge recorded the maximum force experienced during the procedure.

**Statistical Analysis.**

Experiments 1 & 2 – One-way ANOVA with a Dunnett's post-test and regression analysis was performed using GraphPad Prism (version 5.0d for Mac, GraphPad Software, San Diego California USA). All error bars on individual means are reported as the SE of the mean (SEM). Regression error bands are reported as the 95% CI.

Data from Expt. 1 & 2 were used to determine if contact times from different combinations of time and concentration had the same effect on liverwort development. The data were normalized to account for differences in starting values and regressions were made to compare the slopes and elevations between the two.

*Expt. 3:* A two-way ANOVA with a Bonferroni post-test was conducted and complimented by regression analysis. Error bars are reported as the SEM for individual means and as the 95% CI for regressions.

**Results**

*Expt. 1.* The results of Expt. 1 are summarized in Fig. 6.2. Both thallus area and dry matter accumulation were reduced by the treatments beginning at CT 1.25 mg·L⁻¹·min, when compared to the control.
Fig. 6.2: Liverwort thallus area (A) and dry weight (B) accumulation after five consecutive daily applications of aqueous ozone (30 second daily exposure at six aqueous ozone concentrations), followed by a two day recovery period. The ozone treatments are compared to the control using ANOVA with a Dunnet's post-test. Key: NS, *, **, *** Non-significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively; Error bars are +/- the SE.
Expt. 2. Fig. 6. 3 summarizes the thallus area and chlorophyll content response to the ozone treatments. In this experiment, thallus area response was inconclusive (Fig. 6.3A and B); however, chlorophyll content (Fig. 6.3C and D) and dry matter accumulation (Fig. 6.4) exhibited significant negative responses to the application of aqueous ozone. The suppression in dry matter accumulation was uniform across all the ozone treatments (excluding control) and continued through to day 14, a full nine days after treatment cessation (Fig. 6.4).

Contact Time Evaluation. Dry weight data from Expt. 1 and the first harvest of Expt. 2 were compared via regression analysis. The slopes for the two data sets did not differ ($P = 0.2369$), however, the intercepts did ($P < 0.0001$). The difference in the intercept values was used as a correction factor and added to the dry weight values in Expt. 1 to bring those values in line with the values in Expt. 2. The data was then pooled and an overall regression was performed (Fig. 6.5). Based on that regression analysis a toxic threshold CT range was established between CT 0.84 mg·L$^{-1}$·min and CT 1.68 mg·L$^{-1}$·min, which corresponds to a 50-75% reduction in dry matter accumulation (Fig. 6.5); a reduction I consider representative of a response equating to liverwort control. This range is well below the tolerable CT values reported elsewhere for higher plant species (Graham et al., 2009; Sloan and Engelke, 2005).

Expt. 3. The results of Expt. 3 are summarized in Fig. 6.6 – 6.10. The response of liverwort to the 5-applications per week treatment regimes is presented in Fig. 6.6. The zero ozone – low frequency treatment shows healthy tissue with ample gemma cups, whereas the ozone – high frequency application shows significant browning and general tissue damage (Fig. 6.6). Thallus area and dry matter accumulation analysis (Fig. 6.7) confirmed the negative impact on liverwort growth. The 0.0 mg·L$^{-1}$ treatments did not result in growth suppression at different frequencies of application ($P = 0.0692$ (thallus area); $P = 0.5030$ (dry weight)), whereas the ozone treatments did reduce growth at increasing frequencies of application ($P < 0.0001$ (thallus area); $P = 0.0016$ (dry weight) (Fig. 6.7).
Fig. 6.3: Expt. 2 parameter summary: A) Liverwort thallus area accrued after five consecutive days of treatment followed by a two day recovery period; B) after nine days recovery; C) liverwort chlorophyll content index after five consecutive days of treatment followed by a two day recovery period; D) after nine days recovery. Key: ns, *, **, *** non-significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively. Error bars are +/- the SE.
Fig. 6.4: Liverwort thallus dry weight response to aqueous ozone exposure. A summary of the regression analysis conducted for Expt. 2. All non-control ozone treatments were pooled (after individual slope comparison) and a single response relationship presented in comparison to the control regression line. $T_{dw} = \text{Thallus dry weight}$; $T_{dw-p} = \text{Thallus dry weight – pooled}$. Slopes of the two models differ with a $P = 0.0480$. Error bars on individual means are +/- SE; error bars on the regression models are +/- 95% CI.
Fig. 6.5: Regression analysis of pooled data from Expt. 1 and the first harvest of Expt. 2. After correcting for initial dry mass differences, the data were pooled and a best-fit relationship was established. Vertical dashed lines – contact time (CT) damage threshold limits (50-75% reduction in dry matter accumulation). Horizontal solid lines – upper and lower limits of the regression model. Horizontal dashed lines – theoretical dry matter levels representing 50% and 75% reduction over the theoretical maximum dry weight. Error bars are +/- the SE.
Fig. 6.6: Visual comparison of liverwort suppression using aqueous ozone. The experimental units illustrated were both subjected to five treatments per week for 4 weeks. The tissue on the left was treated with tap water while the tissue on the right was exposed to tap water containing 0.5 mg·L⁻¹ aqueous ozone.
Fig. 6.7: Summary of Expt. 3 liverwort thallus area (A) and dry weight (B) response to aqueous ozone treatments (0.0 or 0.5 mg·L⁻¹ for 7.5 minutes) applied at three frequencies (one, three and five times per week) over 28 d. The 0.0 mg·L⁻¹ treatments did not result in growth suppression at different frequencies of application \((P = 0.0692\) (thallus area); \(P = 0.5030\) (dry weight)), whereas the ozone treatments did result in reduced growth \((P < 0.0001\) (thallus area); \(P = 0.0016\) (dry weight)). \(f_a\) = frequency of application; \(A\) = thallus area; \(DW\) = thallus dry weight; Error bars on individual means are +/- SE; error bars on the regression models are +/- 95% CI.
Gemma cups and gemmae production are summarized in Fig. 6.8A - F. Both cup (Fig. 6.8A) and individual gemma (Fig. 6.8D – F) production was reduced by the application of aqueous ozone ($P_{\text{cups}} < 0.0001; P_{\text{gemma}} < 0.0001$), relative to the non-ozonated samples. The degree of reduction also increased with the frequency of ozone application ($P_{\text{cups}} = 0.0001; P_{\text{gemma}} = 0.0195$). The non-ozonated tissue had uniform counts (cups and gemmae) regardless of the application frequency (Fig. 6.8A and B). In addition to reducing the number of cups produced, the ozone treatments damaged the cups (Fig. 6.8B and C). The damage is severe and may impact the ability for water droplets to penetrate the cup structure and dislodge any viable gemmae.

Gemmae size was significantly reduced by aqueous ozone ($P_{\text{day01}} < 0.0001; P_{\text{day07}} = 0.0004$) and the degree of suppression was also dependent on application frequency ($P_{\text{day01}} = 0.0002; P_{\text{day07}} = 0.0005$) (Fig. 9). The average size of the gemmae that received ozone treatments were consistently smaller than the control treatments at all application frequencies (Fig. 6.9A, C and D). There were also significant reductions in gemma size within both concentration regimes as application frequency increased (Fig. 6.9A). The growth of gemmae after treatments ceased was not significantly different when examined proportionally (percentage increase in size)(data not shown); however, the difference in absolute growth (average size of gemmae at day 7) was very significant ($P < 0.0001$) as demonstrated in Fig. 6.9B, E and F.

The force required to remove the thallus tissue from the mineral wool blocks was significantly reduced by the ozone treatment ($P = 0.0009$) and application frequency ($P < 0.0001$) (Fig. 6.10). At an ozone application frequency of five times per week, the removal force was significantly reduced compared to the non-ozone treatment at five applications per week as well as compared to the other ozone treatments at lower application frequencies (Fig. 6.10).
Fig. 6.8: Liverwort gemma cup and gemmae production in response to the aqueous ozone treatments. A) gemma cup development in response to ozone treatments; B) healthy gemma cup with gemmae bodies present (magnification 40x); C) distorted gemma cup as a result of aqueous ozone applications (magnification 40x); D) gemmae production in response to aqueous ozone applications; E) gemma blot from healthy thallus tissue; F) gemma blot from thallus tissue exposed to aqueous ozone at an application frequency of 5x per week. \( f_a \) = frequency of application; \( C \) = cup count; \( G \) = gemmae count. Error bars on individual means are +/- SE; error bars on the regression models are +/- 95% CI.
Graph A: 

- For 0.0 mg·L⁻¹: 
  \[ C_{\text{no-O}_3} = 0.6964 \cdot f_a + 21.20 \]

- For 0.5 mg·L⁻¹: 
  \[ C_{\text{O}_3} = 26.23 - 3.696 \cdot f_a \]

Graph D: 

- For 0.0 mg·L⁻¹: 
  \[ G_{\text{no-O}_3} = 0.536 \cdot f_a + 75.39 \]

- For 0.5 mg·L⁻¹: 
  \[ G_{\text{O}_3} = 71.36 - 12.88 \cdot f_a \]

Frequency of Application (Applications/week)
Fig. 6.9: Summary of gemmae development after cessation of treatments. A) Gemma size at time of transfer to filter disks; B) Gemma size at day 07; C) Representative day 01 selection of gemmae from the 0.0 mg·L⁻¹ – one application per week treatment; D) Representative day 01 selection of gemmae from the 0.5 mg·L⁻¹ – five applications per week treatment; E) Representative day 07 selection of gemmae from the 0.0 mg·L⁻¹ – one application per week treatment; F) Representative day 07 selection of gemmae from the 0.5 mg·L⁻¹ – five applications per week treatment.  \( f_a \) = frequency of application; \( S \) = gemmae size; Error bars on individual means are +/- SE; error bars on the regression models are +/- 95% CI.
Fig. 6.10: Liverwort removal force summary. The average maximum resistive force (newtons) recorded for each treatment regime during the removal of the thallus tissue from the mineral wool growth substrate. Vertical bars within a single frequency level covered by the same horizontal line do not differ at $P \leq 0.05$. Vertical white bars (0.0 mg·L$^{-1}$) do not differ from one another at $P \leq 0.05$. Vertical shaded bars with the same letter do not differ at $P \leq 0.05$. Error bars are +/- the SE.
**DISCUSSION**

The presented study was predicated on the assumption that ozone-containing water is applied directly to nursery or greenhouse crops and that through direct application ancillary benefits, such as liverwort control, may be realized. However, ozonation as an irrigation water treatment technology is often overlooked despite its widespread use in other water treatment applications (Rice, 1999). The oversight is likely the result of well-established phytotoxicity data for ozone gas as a component of photochemical smog (Ashmore, 2005). Operators that have installed ozonation systems typically remove the ozone prior to use, mitigating any risks. The removal is prudent given the paucity of data supporting any other course of action although this limits the effectiveness of the ozone treatment and opens the door for pathogen re-inoculation.

A small body of research has emerged over the last decade that examines irrigation water management strategies involving ozone (Fujiwara and Fujii, 2002; McDonald, 2007; Ohashi-Kaneko et al., 2009; Sloan and Engelke, 2005). Key developments include demonstrations of nutrient stability and improved plant productivity. Graham (2001), and more recently McDonald (2007), and Ohashi-Kaneko et al. (2009) demonstrated that nutrient ions are largely unaffected by the addition of ozone, the exception being iron and manganese which can precipitate during treatment.

Sloan and Engelke (2005), and more recently Graham et al. (2011b) (see Chapters 3 and 4), demonstrated that ozone-containing water applied directly to growth substrates could elicit small stimulatory effects in creeping bentgrass and hydroponic tomato respectively. Sloan and Engelke (2005) suggested that the effect was associated with enhanced nutrient mineralization, while Graham et al. (2011b) further suggested that increased oxygen in the root zone might also play a role.

Graham et al., (2009) (see Chapter 5), demonstrated that foliar application of ozone-containing water is feasible. In that study, ozone-containing water (up to 1.5 mg L\(^{-1}\) at the emitter) did not negatively impact plant productivity or cause visible damage in select woody perennials. The authors did note that off-gas was a concern in overhead irrigation systems and safeguards should be in place to ensure safe conditions for the crop and workers alike. These cautions are reiterated herein.
Before ozone can be used in a plant pest control application, the control parameters need to be reconciled with the desired outcomes. Experiments 1 and 2 confirm (Fig. 6.2 – 6.5) CT as a valid process control parameter. This being said, the CT realized at the crop or plant pest level will differ from that experienced by pathogens in the bulk solution. Measuring the residual ozone concentration and knowing the system residence time will give accurate values for bulk CTs; however, if a solution is being sprayed then the CT realized at the crop will be impacted by pressure changes, drop size, etc. (Fujiwara and Fujii, 2004). Failure to account for these variables would result in unsatisfactory system performance.

Expt. 3 demonstrated that treatments with ozone-containing water, at concentrations safe for select perennial nursery species (Chapter 5), can reduce liverwort development under the conditions examined (Fig. 6.6 and 6.7). Equally, if not more importantly, the study clearly demonstrated a suppression of liverwort asexual fecundity (Fig. 6.8 and 6.9). Ozone treatments reduced the number and size of the gemmae (Fig. 6.8D – E; Fig. 6.9), and although some viable gemmae were observed under the highest frequency and concentration regime, development was delayed (Fig. 6.9). Ozone treatments also damaged the gemma cup structure (Fig. 6.8B and C), which could impact the ability of water droplets to penetrate the cup and dislodge any viable gemmae, further reducing the potential for dissemination.

Increasing application frequency reduced gemma size regardless of ozone concentration (Fig. 6.9). The treatment protocol tended to dislodge mature gemmae, which likely contributed to the observed size reductions. Although the frequency of application influenced the size of gemmae, overall the gemmae from ozone treated liverworts were smaller and less numerous that the non-ozone treatments (Fig. 6.8 D – E; Fig. 6.9A and B). Further, application frequency had no effect on gemma cup numbers or total gemmae produced unless ozone was present, in which case there was a strong reduction (Fig. 6.8).

Expt. 3 also demonstrated that ozone treatments reduced the force required to remove thallus tissue (Fig. 6.10). Given that hand removal is still a common control strategy, the use of aqueous ozone could reduce labor costs and root damage by reducing the substrate binding force that the liverwort exerts (Case et al., 2005; Judge et al., 2004).

Frequency of application is another parameter that needs to be well characterized if a control system is to prove effective and offer flexibility to the grower (i.e. matching watering frequency and pest control requirements). The results presented suggested that a minimum of three treatments in a seven day cycle
(on-going) were required to achieve moderate control, with an application frequency of five times per seven day cycle providing strong control (Fig. 6.6 – 6.10).

The presented data supported the eventual incorporation of ozone-containing irrigation solutions into an overall liverwort management strategy. The use of these ozone-containing solutions for liverwort management is ancillary to the overall objectives of currently installed ozonation systems, which are primarily disease management in the bulk solution. The study has provided baseline application frequencies and CTs required for liverwort control. This said, the study was not representative of, nor should the results be extrapolated to, commercial operational conditions. The lack of background data necessitated tight control over, and high repeatability in, actual liverwort aqueous ozone exposure. This in turn necessitated the use of a dosing system that, although effective at controlling the exposure and providing the requisite repeatability, is not practical for commercial deployment. What the study does provide, in conjunction with other literature cited, is a background for the development of field trials that would confirm or refute these bench scale results. These field trials would also prove useful in elucidating the pre- and post-emergence liverwort management efficacy of aqueous ozone. Extended field trials will be pivotal if ozone-based irrigation water treatment is to move from a batch process incapable of system wide disinfection or direct plant protection, to an in-line process capable of greatly expanded disinfection roles as well as a host of other ancillary benefits.

**CONCLUSIONS**

Contact time appears to be a suitable process control parameter for the regulation of aqueous ozone systems applied to liverwort management. A minimum CT of 0.84 mg·L⁻¹·min is required for control; however, this will vary depending on the nature of the application system. Pre-production trials should be conducted under operational conditions before a hard CT control target is set.

Aqueous ozone has been demonstrated to reduce the growth and fecundity of liverwort, within the limits of crop O₃(aq) tolerance. Therefore, the maintenance of a small residual O₃(aq) concentration during distribution of irrigation water to the crop has the potential to offer some level of liverwort control.

The question mark at the end of the title to this paper is intended to stimulate a scientific exploration of ancillary applications of aqueous ozone used in irrigation water management; applications such as liverwort control. The presented study does not fully answer the question posed, rather it provides a framework from which to expand the knowledge base.
CHAPTER 7

SUMMARY

The conventional operating protocol for using ozone to remediate irrigation water includes the removal of ozone prior to distribution to the crop. This convention has been established based on the best available phytotoxicity data. Unfortunately, those data were based on tropospheric ozone enrichment, a system that is a poor proxy for the application of ozone as an irrigation management tool. Ultimately, the development of horticultural management protocols involving aqueous ozone were built on conjecture, which lead to a situation where the full potential of the technology would be impossible to realize without new research. Although far from resolving all outstanding issues, the work presented has offered a new perspective. In both production systems examined it was demonstrated that aqueous ozone could be applied to select crops without negative growth effects. The irrigation systems examined represent common greenhouse and nursery irrigation strategies. The capacity of diverse crops to tolerate, and in some cases preform better, under aqueous ozone application suggests that the protocols could be broadly employed, although testing should be done to ensure each crop’s tolerance to aqueous ozone.

Demonstrating aqueous ozone – crop compatibility was the first major goal of the research. Having established this compatibility, the question then became one of developing ancillary benefits now that ozone can remain in solution during irrigation. There has been a great deal of work done on pathogen control with ozone, and in all likelihood the maintenance of residual ozone will improve system-wide hygiene. Although a worthy research avenue, I was more interested in plant pests. Liverwort control posed a particularly interesting scenario. Much of the thesis was spent showing that plants can tolerate aqueous ozone. Once this was established, the objectives became quite the opposite. I now wanted to demonstrate phytotoxicity in a plant pest species. The key would be in achieving a measure of control in one species (i.e. liverwort) within the tolerance limits of another species (i.e. crop species). There are several physical characteristics of liverwort, such as, lack of guard cells to control a stomata aperture, which suggested control might be possible within the ozone tolerance limits of the crop species examined. The study conducted (Chapter 6) clearly demonstrated that control within the tolerance limits of the crop is possible, although a commercially relevant application strategy still needs to be examined.
It is still premature to recommend that growers apply aqueous ozone solutions to their crops as described in this thesis. There are still numerous outstanding questions regarding the applications developed; however, there is now sufficient evidence to justify the development of large-scale field trials. These field trials should be designed to provide growers with the operational and horticultural management protocols that will ultimately prove the technology and its applications in a commercial setting. Further, these trials would prove valuable for expanding the list of species and cultivars that are amenable to aqueous ozone applications.

**GOING FORWARD**

**EXPAND THE SCOPE**

The material presented provides a solid baseline from which future research can build. The obvious next step in further developing the applications established herein is to expand the number of crop species and types of production systems examined. There will undoubtedly be crops that are less tolerant to the application of aqueous ozone than those examined in the present work. Further, the irrigation delivery system utilized may have an influence on the compatibility of ozonation with crop production. Producers employing the nutrient film technique (NFT) for example may find that phytotoxic thresholds are lower than those examined in the work presented. In NFT systems, the direct root exposure to aqueous ozone would be greater than in the systems currently examined and as such the technologies (NFT and ozonation) may not be compatible; further research is needed to address such compatibility issues.

**PLANT DEFENSES – SYSTEMIC ACQUIRED RESISTANCE AND SMOG IMMUNIZATION**

Although a weak proxy for ozone-based irrigation management technologies, research on tropospheric ozone effects on plant production has shed light on aspects of plant physiological responses that may be of use in the development of relevant applications for these systems. Numerous ozone enrichment studies have examined the apparent similarities between plant responses to a pathogen challenge and the response to an ozone exposure (Sandermann et al., 1998; Sandermann, 1996; Kangasjärvi, 1994; Mahalingam et al, 2003; Sharma et al., 1996). It was recognized that ozone could stimulate the development of systemic acquired resistance (SAR) (Sudhakar et al., 2007; Sandermann et al., 1998; Sharma et al., 1996; Kangasjärvi, 1994); therefore, ozone exposure may improve disease resistance under some circumstances. Given this, it would be interesting to examine the potential of using aqueous ozone not only as part of an irrigation management strategy, but also as part of a broader pathogen prevention plan.
In a similar fashion to the stimulation of SAR, there may be a role for using aqueous ozone as a tool for ‘immunizing’ crops against ozone exposure resulting from photochemical smog events. Exposure to ozone has been shown to stimulate antioxidant pathways in some plant species (Lyons and Barnes, 1998; Sandermann, 1996; Walmsley et al., 1980; Mahalingam et al, 2003; Conklin and Last, 1995). Provided that the ozone dose is tightly controlled, which is possible using aqueous exposure systems, it may be possible to stimulate these responses early in the crop cycle during periods of lower background ozone levels (e.g. spring and early summer in Ontario). Charging up the plants ability to defend against ozone and associated free radicals in advance of significant smog events may mitigate the damage typically associated with these events. This would be particularly useful in floriculture crops where the value of the commodity is entirely based on how it looks (cf. vegetable crops).

**Final Thoughts**

Although the presented work opens the door for a greatly expanded role for ozone in irrigation solution management, ozonation is by no means a comprehensive solution. Maintaining water quality is difficult and any attempt to address the issue of irrigation solution management will undoubtedly require multiple technologies and management practices combined in an integrated solution management (ISM) plan. It is hoped that the presented work will provide others with the baseline information required to develop new ISM protocols that include ozonation technology where it is appropriate.
LITERATURE CITED


McDonald, G.V. 2007. Ozone (O3) efficacy on reduction of Phytophthora capsici in recirculated horticultural irrigation water. Texas A&M, College Station, PhD Diss. pp. 121.


APPENDICES

APPENDIX 1

INTRODUCTION

Ozone (O₃) is a molecule of relatively simple structure, being the tri-atomic allotrope of oxygen; however, this simple structure belies its complex chemistry and involvement in many life-critical (life as we know it anyway) reactions. Ozone plays many roles in the 'normal' operation of Earth's atmosphere, from a phytotoxic (as well as human) by-product of the interaction of solar radiation and 'industrial' emissions, to a critical life-enabling role in the interception of UVC and UVB radiation in the stratosphere. Over the past 30-40 years a figurative mountain of research has been conducted on these two aspects of ozone chemistry alone, yet there are many questions still requiring the attention of scientists around the world. In the shadow of this mountain, there are also many other applications of ozone that have received significant research attention, while there are yet others that have not been given full due.

I could easily spend a page or more just listing the various applications of ozone, such as its use as a sterilant in some types of brain surgery to the disinfection of many of the world's municipal water supplies; however, these applications are not the focus of this discussion. Rather, the discussion presented will focus on: 1) The thermodynamics of the system outlined in equation 0.1; 2) The major influencing factors on this system; and 3) How these relationships and factors dictate the applicability of aqueous ozone (O₃(aq)) in various agricultural (plant production) operations.

\[ \text{O}_3(g) \rightleftharpoons \text{O}_3(aq) \]  

Equation 0.1

It was stated earlier that the simplicity of the ozone molecule belies its complex chemistry. Likewise, the simplicity of equation 0.1 conceals the complex nature of ozone solubility and mass transfer dynamics in aqueous solution. A portion of the complexity is derived from the end applications that drive the chemistry. It is true that in pure water, under the right conditions (low pH), the system is relatively simple; however, the overwhelming majority of all applications involve ozone dissolution into solvents that are chemically complex. These complex solutions can dramatically alter the solubility of ozone and must be taken into account when developing an ozone application. In addition to these often unknown or poorly understood conditions, or perhaps more correctly the unknown influence of these conditions, there are also physical considerations such as temperature and system pressure that also play a significant role in O₃ solubility.

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14 Not to pass the buck -- we are ALL responsible for these emissions and cannot simply blame it on 'Big Industry'.
15 Many of these applications and the chemistries involved are fascinating and may provide fodder for discussion during the oral exam.
16 In the cases relevant to this discussion, the solvent is water plus all the other 'stuff' dissolved or suspended in it.
In reviewing the literature for this discussion it quickly became clear that the verdict is still out on just what is and isn't important when considering ozone solubility, and more importantly that this system is far from 'figured out'. I will admit that initially I was naive and thought, 'how hard can it be?' There is a gas and you are dissolving it in water, a pretty routine situation! I humbly stand corrected; it is not so easy and the many nuances of the system continue to contribute to the retreat of my hairline. The following discussion starts with the basic thermodynamics of the system and works up, and sometimes around, the various considerations that need to be taken into account when examining ozone solubility for application development. The discussion will then venture into the realm of agricultural applications of ozone, specifically the use of ozone for the remediation of irrigation and fertigation water resources and the potential to use ozone as a plant protectant. The primary focus of that particular discussion will be, 'What happens, in terms of thermodynamics and mass transfer, when you spray O$_3$(aq)'. So let's begin.

1. OZONE MASS TRANSFER & SOLUBILITY

BASIC THERMODYNAMICS OF THE GAS ⇄ WATER SYSTEM

The dissolution of gases into liquids has been the subject of untold volumes of research, both empirical and theoretical; research that has resulted in numerous theories and equations to model the solubility and mass transfer of a gas into a liquid. I do not presume to fully grasp the complexities of all these theories or solution chemistry as it pertains to the dissolution of a gas in a liquid; however, a basic understanding of aqueous solution chemistry is key to the research that I have become involved with. I will begin this discussion then with a general overview of the dissolution of a gas (the solute for this discussion) in water (the solvent for this discussion). In order to keep things simple, I am assuming that the gas is ideal, the solvent is pure water, and the system is at standard temperature and pressure (STP).

There are many ways to calculate and examine gas solubility, some of which will be discussed later in this document. The basis for all the methods however can ultimately be linked back to the basic thermodynamics that govern the system. Therefore, I will begin with a discussion of the energetics of gas dissolution.

GIBBS-HELMHOLTZ – UNITING THE GOVERNING FUNCTIONS OF THE SYSTEM

The Gibbs-Helmholtz relationship (equation 1.1) is a key relationship in physical chemistry, as it allows for the prediction of spontaneity, and hence direction, of a reaction or process. The Gibbs-Helmholtz relation links the enthalpic (H) and entropic (S) contributions to a process to give the overall free energy change (ΔG) for the process.

\[ ΔG = ΔH - TΔS \]

Equation 1.1

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17 Fertigation is a term used to describe irrigation with fertilizer added to the water. Primarily a term used in the greenhouse industry.
18 For the purposes of the discussion, the gas strictly follows the Ideal Gas Law PV=nRT and is non-reacting.
19 There are many variations and relations that relate to the form of the equation expressed in equation 2
The sign of $\Delta G$ determines the spontaneity (and direction) of the process, with values of $\Delta G < 0$ being thermodynamically favourable (spontaneous), $\Delta G > 0$ being unfavourable (non-spontaneous), and $\Delta G = 0$ being a process that is has reached equilibrium (all else being equal, no further overall compositional change will occur). It should be noted that just because a process is thermodynamically favourable does not imply that it will proceed at an appreciable (or even perceptible) rate. The rate of the process will be governed by the reaction rate constant for the process at a given temperature. 

The influence of temperature not only affects the rate of the process, it also has an impact on the spontaneity of the process as is evident from equation 1.1. Recall that for a process to be spontaneous, $\Delta G$ needs to be negative. Equation 1.1 then dictates that for a process to be spontaneous, the combined enthalpic and entropic terms must be negative. The negative requirement can be achieved even if the enthalpic component ($\Delta H$) is positive providing that $\Delta S$ is positive and the temperature of the system is high enough to make $|T \Delta S| > \Delta H$. Basically, a process can be non-spontaneous at low temperatures, becoming spontaneous as the temperature rises. Conversely, the reverse situation would result in a system that is spontaneous at low temperatures, becoming non-spontaneous as the temperature rises. This second case is relevant to the understanding of the higher solubility of gases at lower temperatures and will be discussed further in coming sections.

**Thermodynamic Equilibrium Constant ($K$) and its Relation to $\Delta G$ – A Key Concept in Understanding Solubility Equilibria**

The free energy of a system can be expressed in many ways by relating $\Delta G$ to other parameters. One such relation is expressed in equations 1.2 and 1.3, which relate the standard molar free energy change $\Delta G^0$ and $\Delta G$ to the thermodynamic equilibrium constant ($K$) at a given temperature.

$$\Delta G^0 = -RT \ln K$$  \hspace{1cm} \text{Equation 1.2}

The value for $\Delta G^0$ can be obtained from standard tables for the components of the process in question. The conditions required for $\Delta G^0$ are rarely encountered in a 'real life' situation, and it would be handy to relate $K$ to a more useful parameter. The $\Delta G$ can be calculated for 'real' conditions by replacing $K$ with the reaction quotient $Q$ and using the tabulated values of $\Delta G^0$ to determine $\Delta G$. The relationship is outlined in equation 1.3.

$$\Delta G = \Delta G^0 + RT \ln Q$$  \hspace{1cm} \text{Equation 1.3}

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20 The free energy change occurring when each reactant and product species is present at unit activity (~STP) – Bunce (1993)  
21 All species are present at unit activity (~1 mol L$^{-1}$ for solutions and 1 atm for gases)  
22 Is the relative proportion of the system products and reactant at a given point in time during the process/reaction
A useful (and explanatory) relationship can be developed by combining the standard molar version of equation 1.1 (i.e. Replace $\Delta G$, $\Delta H$, and $\Delta S$ with $\Delta G^O$, $\Delta H^O$, and $\Delta S^O$) and equation 1.2 while isolating the ln$K$ term (equation 1.4).

$$\ln K = -(\Delta H^O/RT) + (\Delta S^O/R)$$ \hspace{1cm} \text{Equation 1.4}

It is can be shown from this relationship that $K$, at a given temperature, is dependant only on the sign and magnitude of $\Delta H^O$. The $\Delta S^O/R$ term is a constant, whereas the $\Delta H^O/RT$ term varies with temperature (T), which in turn means that $K$ varies with temperature in relation to the value of $\Delta H^O$.

At this point I need to introduce the concepts associated with the solubility equilibrium constant (K), and more specifically Henry's Law, but I will return to this discussion to further explain gas solubility on a thermodynamic basis.

**HENRY'S LAW – DESCRIBING THE EQUILIBRIUM SOLUBILITY OF A GAS IN WATER**

Henry's Law describes the equilibrium distribution, for some molecule or compound, between the bulk liquid and gas phase. Essentially, Henry's Laws states that the concentration of the gas in solution is proportional to the concentration (partial pressure) of the gas in contact with the solution. In order to keep things simple, this relationship is for dilute, non-reacting solutions, of which neither typically apply to common aqueous ozone solutions, but I will deal with that later. The relationship can be expressed mathematically by equation 1.5.

$$[X_{(aq)}] = K_H \cdot p(X_{(g)})$$ \hspace{1cm} \text{Equation 1.5}

where;  
$[X_{(aq)}]$ is the gas concentration in water with units of mol L$^{-1}$ (or equivalent)  
$K_H$ is the Henry's Law/Equilibrium constant with units of mol L$^{-1}$ atm$^{-1}$ (or equivalent)  
$p(X_{(g)})$ is the partial pressure of the gas with units of atmosphere (or equivalent)

Rearranging equation 1.5 gives the expression of $K_H$ (equation 1.6):

$$K_H = [X_{(aq)}]/p(X_{(g)})$$ \hspace{1cm} \text{Equation 1.6}

Example: A typical small-scale ozone generator fed with pure oxygen at STP produces an output of 0.08 atm. What is the equilibrium concentration of ozone in the aqueous phase if the gas is allowed to equilibrate with a fixed volume of pure water? Note: the $K_H$ value given is taken from Bunce (1993), however, the value is still a point of debate in the literature.

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23 Valid for any solvent, but I am focusing on water  
24 Nitrogen gas is typically added (2-5%) to improve ozone production
\[
\text{[O}_3\text{(aq)]} = 1.3 \times 10^{-2} \text{ mol L}^{-1} \cdot \text{ atm}^{-1} \cdot 0.08 \text{ atm} \\
= 1.04 \times 10^{-3} \text{ mol L}^{-1}
\]

converting to mg/L:
\[
\text{[O}_3\text{(aq)]} = 1.04 \times 10^{-3} \text{ mol L}^{-1} \cdot 48 \text{ g mol}^{-1} \cdot 1000\text{ mg g}^{-1} \\
= 50 \text{ mg L}^{-1}
\]

I would like to note at this point that there are additional parameters for establishing the solubility of an ozone-water system, namely the Bunsen coefficient (\(\beta\)) and the Solubility Ratio (S). The Bunsen coefficient is defined as the ratio of the volume of ozone at STP dissolved per volume of water, with the condition that the partial pressure of O\(_3\) is 1 atm. The Solubility Ratio is the quotient between equilibrium [O\(_3\)\text{(aq)] and [O\(_3\)\text{(g)]}. The three parameters are related to one another by equation 1.7.

\[
K_H = 8039(T/S) = 2268373(1/\beta)
\]

Equation 1.7

Now let's get back to the thermodynamic discussion of solubility!

\textit{Equation 1.4} relates the thermodynamic equilibrium constant (\(K\)) to the thermodynamic properties of the system (\(\Delta H^O\) and \(\Delta S^O\)). Conveniently (when the fugacity coefficients are \(\sim 1\)), \(K\) is numerically equal to \(K_H\) which allows us to relate the solubility of a gas to thermodynamic parameters via \textit{equation 1.4}. This numerical approximation allows us to easily estimate the influencing factors on solubility.

\textbf{Why are most common gases only sparingly soluble in water?}

The relationship outlined in \textit{equation 1.4} offers insight into the reasons that many of the gases that we are familiar with, including ozone, are only somewhat soluble under normal conditions. First, it should be noted that the \(\Delta H^O\) associated with the dissolution of all gases is negative (exothermic), which is favourable (contributes favourably to producing a negative \(\Delta G\)) in terms of dissolution. If things were left there, then all gases would be very soluble, but they are not. The reason for the low solubility then must be dependant on the entropy component, as it is the only other factor influencing the equilibrium constant in \textit{equation 1.4}.

When a gas molecule enters into solution, its movements are greatly restricted compared to the gas phase. This restriction on movement translates into an increase in system order, which is a positive contribution to the entropy term. A second 'ordering' occurs when the gas molecule enters solution. The water molecules tend to form a 'sheath' around the gas molecule. The creation of these 'sheaths' represents an ordering of the solvent. The combination of these two ordering mechanisms results in a very unfavourable \(\Delta S\) component, which tends to counteract the favourable \(\Delta H\).
Why does solubility decrease with increasing solution temperature?

The decreased solubility associated with an increase in temperature (for all gases) can be explained by examining the structure of equation 1.4, which may be clearer for this discussion if written in exponential form (equation 1.8).

\[ K = e^{-(\Delta H_0/RT)} \cdot e^{+\Delta S_0/R} = e^{-(\Delta H_0/RT)} \cdot \text{constant} \]

Equation 1.8

I mentioned previously that the enthalpic term is favourable for the dissolution of gases into water, as the process in universally exothermic. The exothermic nature of the dissolution of gases results in a positive exponent term that decreases as the temperature (T) gets larger. The result is a decrease in the value of the equilibrium constant (K) as temperature increases. To state the obvious, the lower equilibrium constant results is a lower gas solubility as the temperature is increased. The literature is littered with graphs describing the temperature dependence of ozone gas solubility and will not be reproduced here.

Salt Content Influences Ozone Solubility

It is well known that the presence of inorganic ions (salts) affect the solubility of gases in solution, both decreasing (salting-out) the solubility as well as increasing the solubility (salting-in) in some cases. In the case of ozone-water systems, the effect is typically that of salting-out, but there are a few important instances of salting-in as well. Ozone – water systems are typically characterized by relatively low [O₃(aq)], ambient pressure and temperature. Applications running under these conditions still typically look to maximize ozone solubility and mass transfer, therefore the influence of dissolved salts requires consideration.

The influence of ionic components on the solubility of a gas are described by the Sechenov Equation (Equations 1.9), which relates the influence of overall ionic strength and the make up of the ion and gas mix to an 'apparent' Henry's Law constant (K_{H-app}).

\[ K_{H-app} = K_{H-sfw} \cdot 10^{K_s c_s} \]

Equation 1.9

where:
- \( K_{H-app} \) is the apparent Henry's constant
- \( K_{H-sfw} \) is the Henry's constant in salt-free water
- \( K_s \) is the Sechenov constant specific to the gas and salt
- \( c_s \) is the concentration of the salt

Over the years many improvements have been made to the basic Sechenov equation; improvements that take into account unknown values of \( K_s \), the contributions of both gas and ions towards \( K_s \) values, and wider provide estimates over wider temperature ranges.

Sotelo et al (1989) developed Henry's Law constant equations for a range of buffer solutions commonly used in O₃(aq) studies. The group established equations for \( K_{H} \) that cover a temperature range of 0-20ºC, a pH range of 2-8.5, and an ionic strength of 10⁻¹ to 10⁻³. These equations
focused largely on sodium salts and as such are not necessarily applicable to nutrient solution solubility calculations, but they do provide a starting point from which to develop empirical relationships for nutrient solution ozone solubility.

2. Mass Transfer – What is Going on at the Gas – Liquid Interface?

Mass transfer, as the name implies, is simply a transfer of a substance (although the concepts also apply to heat and momentum) from a high concentration to a low concentration through a variety of diffusive and convective processes. When the system in question is a gas – liquid interface, then several resistive components to diffusion must be taken into account. As is the case with many complex systems, assumptions are made to 'tame' the math and to allow for actual solutions to be calculated.

Fick's Law of Diffusion

Fick's Law (specifically his first law) (equation 2.1) relates the mass transfer flux ($J$) of a substance to the concentration field (the general concentration patterns or states of a system) by suggesting that a substance will move from a zone of high concentration (of that substance) to an area of low concentration (of that substance). The law also suggests that the magnitude of the flux will be proportional to the magnitude of the concentration gradient between the zones of high and low concentration.

$$J = -D \cdot (\Delta C/\Delta x)$$  \hspace{1cm} \textit{Equation 2.1}

where:  
$J$ is the mass flux (mole time$^{-1}$ area$^{-1}$)  
$D$ is the diffusion coefficient (diffusivity) (area time$^{-1}$)  
$C$ is the concentration of the diffusing substance (mole volume$^{-1}$)  
$x$ is the distance the substance is diffusing (length)

The relationship outlined in equation 2.1 is relatively straight forward, but it does not take into account the complexities of turbulent flow, reactions of the diffusing compound, and many other 'real world' influences. This being said, it does give very good estimates for all but the most precise work. The only limitation that this version of Fick's Law presents is that it doesn't directly explain the movement of the compound from one medium (gas) to another (water for argument sake). This can be overcome by any of the phase transition models available. A general model is the Two-Film model put forth by Lewis and Whitman in 1924 (with improvements being made over the ensuing years).

Two-Film Theory of Mass Transfer

In its most simplistic interpretation the two-film theory is Fick's Law times two plus an interaction or interface component. The general concept is outlined in Fig. 1, with each 'half' of the diagram representing a compartmentalized occurrence of Fick's Law, with the 'interface' representing the third, linking component. The presented model makes the following assumptions about the system: 1) The bulk solutions are 'well mixed' and the concentrations are uniform (i.e. If the bulk concentrations do change, it is slow enough not to impact the processes occurring in the two films and interface component
at any given time point); 2) The phase interface is a sharply defined boundary; 3) The 'film' on either side of the interface is laminar; 4) The laminar sections are sufficiently thin to assume steady state diffusion; and 5) The interface is in a steady state condition and is therefore in equilibrium with both film layers.

Based on Fick's Law and considering the additional components outlined in Fig. 1, a mass transfer coefficient can be calculated for each phase. Equations 2.2 through 2.5 summarize the relationships.

\[ k_{gf} = \frac{D_g}{x_g} \quad \text{Equation 2.2} \]
\[ J = k_{gf} \cdot (C_g - C_{gi}) \quad \text{Equation 2.3} \]

where:
- \( k_{gf} \) is mass transfer coefficient for the gas phase
- \( D_g \) is the diffusivity of the gas in the gas phase
- \( x_g \) is the distance over which the diffusion is taking place
- \( J \) is the diffusive flux
- \( C_g \) is the concentration of the 'solute gas'
- \( C_{gi} \) is the concentration at the gas phase film – interface junction

\[ k_{wf} = \frac{D_w}{x_w} \quad \text{Equation 2.4} \]
\[ J = k_{wf} \cdot (C_w - C_{wi}) \quad \text{Equation 2.5} \]

where:
- \( k_{wf} \) is mass transfer coefficient for the liquid phase
- \( D_w \) is the diffusivity of the gas in water
- \( x_w \) is the distance over which the diffusion is taking place
- \( J \) is the diffusive flux
- \( C_w \) is the concentration of the 'solute gas'
- \( C_{wi} \) is the concentration at the water phase film – interface junction

The mass transfer model outlined in Fig. 1 is also valid (when reversed) with the gas concentration being greater in the liquid phase, which is an important process when considering the movement of a gas out of solution as would be the case when \( O_3(aq) \) is sprayed on a crop or commodity.

Although an effective model for gas – liquid systems, the basic two-film model is limited by its assumptions. Two additional and progressive theories have been developed from the original model put forth by Lewis and Whitman (1924), namely the Penetration Theory (Higbie, 1935) and the Surface Renewal Theory (Dankwerts, 1951). I will briefly discuss these theories with respect to the 'improvements' they offer over the original Lewis and Whitman (1924) model in terms of understanding, although the original model is still often employed due to its relative ease of use.

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25 The most widely used version of the Surface Renewal Theory
Fig. A1.1: Graphical representation of the Two-Film Theory of mass transfer from a gas to a liquid, based on the original work of Lewis and Whitman (1924). In the model, gas moves from the 'well mixed' bulk phase into the gas thin film. The diffusion through the film is governed by Fick's Law and is assumed to be steady state. The gas then passes through the gas-liquid interface and enters into another diffusion regime within the liquid film, again diffusion is assumed to be steady state and governed by Fick's Law. The gas solute then reaches the bulk liquid, where the solute concentration is assumed to be uniform (bulk solution 'well mixed').

Note: If the liquid (or constituents of the liquid) are reactive with the dissolving gas, then several kinetic regimes can occur in the liquid film layer, from instantaneous reaction (no gas reaches bulk solution) to slow/no reaction (all diffusing gas reaches bulk solution—shown here).
**Penetration & Surface Renewal Modifications to the Two-Film Theory**

In many gas dissolution apparatus, the turbulence is extremely high, which can cause the actual contact time between discrete parcels of gas (bubbles) and liquid (droplets) to be so short that the assumption of steady state cannot be justified. In the Penetration Theory, Higbie (1935) suggests that the mass transfer coefficient is inversely proportional to the square root of the contact time and is given in equation 2.6.

\[
k_{\text{mean}} = \frac{2}{\sqrt{\pi}} \cdot \left(\sqrt{D/t}\right)
\]

Equation 2.6

where: 
- \(k_{\text{mean}}\) is the mean mass transfer coefficient
- \(t\) is the contact time at the mass transfer interface

The Penetration Theory assumes that the contact times are uniform throughout the mixing apparatus, which is unlikely to be the case, but it is a reasonable simplification. The Surface Renewal Theory takes the Penetration Theory one step further and employs a temporal distribution function to describe the contact times between individual parcels. The parcels are in contact for a period of time (which can vary) and then move out of contact to be replaced by another parcel at the contact surface (this parcel renewal at the transferring surface is the name sake of the theory) which may or may not maintain contact for the same amount of time as the previous parcel.

Up to this point, it has been assumed that the dissolving gas is non-reacting and that the solvent was pure water. Ozone systems are anything but non-reactive except under the most restrictive of conditions. Typical calculations of ozone solubility are for solutions that contain a wide range of 'contaminants' and other factors that influence the mass transfer and stability of the ozone in solution. The major factors governing or altering the theoretical solubility will be discussed with at focus on the factors that are most likely to be relevant to an agricultural application.

**Stability of Ozone in Solution**

Let me start of this section by stating that ozone is not stable in aqueous solution, at least for the situations that would be encountered in agricultural water use applications. The basis for this statement is the fact that ozone is extremely reactive, and once in solution it is subject to countless decomposition reactions. The primary influencing factors on \(O_3(\text{aq})\) stability are:

1) Temperature
2) pH and alkalinity
3) Contaminant concentration and composition

This is by no means an exhaustive list of contributing factors to the stability of ozone in aqueous solution, but it does represent the factors with potentially the largest magnitude of influence on the stability of ozone in solution when considering agricultural applications.
**Temperature**

The effect of temperature on ozone solubility in aqueous solution is the same as that for any other gas – liquid system. Increasing temperature decreases solubility as was discussed in a previous section. Temperature also has an influence on rates of reactions through its contribution to the value of the reaction rate constant (k) for a reaction system. The Arrhenius Equation \((equation \ 2.7)\) implies that as temperature increases the exponential factor becomes larger (less negative), which results in an increase in the rate constant.

\[
k = A \cdot e^{-\frac{E_a}{RT}}
\]

where:
- \(k\) is the rate constant
- \(A\) is the pre-exponent factor/constant
- \(E_a\) is the activation energy for the reaction
- \(R\) is the gas constant
- \(T\) is the temperature of the system

The typical operating temperature range for the agricultural applications of ozone being studied are relatively small (10-25°C). Depending on other prevailing conditions the influence of temperature may be small in comparison to other contributing solubility (or mass transfer) factors. This being said, the influence of temperature should not be discounted when considering the effectiveness of pathogen control in a treatment stream. In biological systems the influence of temperature on ozone reaction rates with bio-molecules, at the temperatures encountered in agricultural systems could be a very significant contributor to overall system efficacy.

**Hydroxide Ions and Alkalinity**

The pH of the solvent solution is perhaps one of the most significant influencing factors on the actual stability of ozone in solution. Specifically, it is the hydroxide ion \([OH^-]\) that is of primary importance. In order to better understand the influence of hydroxide ions on \(O_3(aq)\) stability, it is worth discussing the larger picture of ozone decomposition.

**Initiators, Promoters and Terminators of Ozone Decomposition Reactions**

Ozone decomposition reactions in large part govern the stability of ozone in relatively clean waters (contaminant reactions become more important when the solution is heavily degraded). Although the decomposition reactions reduce the stability of ozone in aqueous solution, these same reactions are critical for many of the so called Advanced Oxidation Processes (AOP). In these AOP's the breakdown of ozone in solution generates hydroxyl radicals which can react with recalcitrant or ozone insensitive compounds, which enhances the overall effectiveness of many treatment processes.

The heading of this section indicates that there are three classes of compounds that influence the decomposition rate of ozone in solution: Initiators, which trigger the initial breakdown step (may or may not be involved in additional reactions); Promoters, which act to generate further decomposition-supporting compounds; and Terminators, which essentially scavenge the radical
species that act as promoters. The combined reactions of this decomposition system are far too numerous to be dealt with here; however, a simplified mechanism is presented for the OH\(^-\) initiated mechanism. This mechanism highlights the key role that pH plays in the stability of ozone in aqueous solution.

**Initiation**

The hydroxide ion (OH\(^-\)) reacts with Ozone to generate the superoxide anion radical (O\(_2^\cdot\)) and the hydroperoxyl radical (HO\(_2\)\(^\cdot\)) (equation 2.8), which play a key initiation role in the radical chain reactions that promote further decomposition.

\[
O_3 + OH^- \rightarrow O_2^\cdot^- + HO_2^\cdot \quad k = 70 \text{ M}^{-1}\text{s}^{-1} \quad Equation 2.8
\]

\[
HO_2 \rightleftharpoons O_2^\cdot^- + H^+ \quad pK_a = 4.8 \quad Equation 2.9
\]

**Promotion – Radical Chain Reactions**

The ozonide anion radical (O\(_3^\cdot\)) is formed by the reaction of ozone with the superoxide radical (equation 2.10), which quickly leads to the formation of hydroxyl radicals (OH\(^\cdot\)) (equation 2.12).

\[
O_3 + O_2^\cdot^- \rightarrow O_3^\cdot^- + O_2 \quad k_2 = 1.6 \times 10^9 \text{ M}^{-1}\text{s}^{-1} \quad Equation 2.10
\]

\[
HO_3^- \rightleftharpoons O_3^- + H^+ \quad pK_a = 6.2 \quad Equation 2.11
\]

\[
HO_3^- \rightarrow OH^- + O_2 \quad k_3 = 1.1 \times 10^8 \text{ M}^{-1}\text{s}^{-1} \quad Equation 2.12
\]

The hydroxyl radical can then react with ozone, leading once again to the hydroperoxyl radical (equations 2.13 and 2.14) which continues the radical-based decomposition chain reaction.

\[
OH^- + O_3 \rightarrow HO_4^- \quad k_4 = 2.0 \times 10^9 \text{ M}^{-1}\text{s}^{-1} \quad Equation 2.13
\]

\[
HO_4^- \rightarrow O_2 + HO_2^\cdot \quad k_5 = 2.8 \times 10^4 \text{ M}^{-1}\text{s}^{-1} \quad Equation 2.14
\]

It can be elucidated from equations 2.8 through 2.14 that any substance that generates \(\text{HO}_2^\cdot\) or \(O_2^-\) during its reaction with \(\text{OH}^-\) will promote the chain reaction and can be considered a promoter of ozone decomposition in aqueous solution.

**Termination – Scavengers**

Some substances that react with the hydroxyl radical to form secondary radicals other than \(\text{HO}_2^\cdot\) and \(O_2^-\) can terminate the radical chain reactions, thereby stabilizing ozone decomposition to some degree. A second common chain terminating reaction is that of \(\text{OH}^-\) with another radical. If the second radical is the hydroperoxyl radical, then the termination step is all the more effective (equation 2.15).
A common and important OH⁻ scavenger system in natural waters (particularly around Guelph), is the carbonate/bicarbonate ion complex. The basic reactions are shown in equations 2.16 and 2.17.

\[
\text{OH}^- + \text{CO}_3^{2-} \rightarrow \text{OH}^- + \text{CO}_3^- \quad k_1 = 4.2 \times 10^8 \text{ M}^{-1}\text{s}^{-1} \quad \text{Equation 2.16}
\]

\[
\text{OH}^- + \text{HCO}_3^- \rightarrow \text{OH}^- + \text{HCO}_3^- \quad k_2 = 1.5 \times 10^7 \text{ M}^{-1}\text{s}^{-1} \quad \text{Equation 2.17}
\]

Carbonate is the stronger scavenger in this system (compare rate constants), but both do contribute significantly to the stabilization of ozone in solution. Hoigne and Bader (1977) demonstrated that even very small additions of carbonate to an ozone solution could reduce the decay rate by an order of magnitude.

Equations 2.8 through 2.17 outline the basic governing reactions that relate pH and alkalinity to the stability of ozone in aqueous solution. These same reactions also highlight the importance of defining and considering pH and alkalinity of source waters when developing specific ozone applications.

3. AGRICULTURAL APPLICATION CONSIDERATIONS

The research that I have undertaken is examining the potential of using ozone to control disease and plant pests in greenhouse and nursery irrigation and fertigation systems. The assumption is that given proper system design, ozone can be effective for the control of organic contaminants and pathogens in bulk solution (i.e. ‘in the tank’ systems). This is a common treatment option in many other industries with equally or greater contaminant loads in the source water. The innovation that I am exploring revolves around leaving a residual [O₃(aq)] in the water as it is distributed to the crop. The concept is to treat the entire distribution system, including the plants themselves. The theory is that low-dose aqueous ozone sprayed on the crop may act as a protectant (pollution and pathogens) and potentially control the plant pest *Marchantia polymorpha*. There are many factors to consider when contemplating this particular application of O₃(aq), but two critical factors that are relevant to the current discussion are the effects of fertilizer salts on the solubility and stability of aqueous ozone solutions, and the losses of ozone from solution when the irrigation/fertigation solution is sprayed on the crop.

FERTILIZER SALTS AND OZONE SOLUBILITY

There are many studies that have investigated the influence of individual salts on the solubility of ozone. Some studies have also attempted to evaluate and model the influence of a mixture of salts on gas solubility. In general, these studies have examined the solubility effects at relatively high salt concentrations (1.0M and greater levels). It is generally accepted that dilute solutions have only minor impacts on gas solubility and, except for precise work, can often be ignored for most common ions. The
question then becomes, 'What is a dilute solution, and where does a 'typical' hydroponic nutrient solution fall in the gradient?'

Generally speaking, a dilute solution is one in which the sum of the amount fractions (the amount of substance actually dissolved) is small relative to the total amount that could be dissolved under the same conditions. Hydroponic solutions are ioniically complex, with 10-16 different salts added to the solution at concentrations ranging from a few hundred mg L\(^{-1}\) down to fractions of a mg L\(^{-1}\) for the micro nutrients. Although complex, and often applied at concentrations greater than is physiologically necessary, the solutions are still very dilute in order to maintain an optimal osmotic balance with the roots (to allow water uptake). Many nutrient solution recipes have been suggested over the years, with many being tailored to the specific requirements of a selected crop. A common solution used for research purposes, often with modifications, is that proposed by Hoagland (1938). The general make up of this solution, on an elemental basis, is presented in Table 1.

Hydroponic solutions used for periodic or pulse-feed fertigation systems are often an order of magnitude more concentrated than soil solutions, to ensure that sufficient nutrients are delivered to the plant during the watering phase. The Hoagland solution presented in Table 1 is of such magnitude; however, many researchers opt to reduce these concentrations by 50%, reporting the use of half-strength Hoagland solutions in their methodologies. Several hydroponic systems have been developed to better approach soil solution concentrations. These systems typically have a continuous flow through the root zone to ensure a constant supply of nutrients. In these systems, the concentrations are considerably lower (Table 2) than in pulsed fed systems.

In general, the salting out effect can be predicted by complex computations; however, the only reliable method to evaluate the influence of a complex salt solution on the solubility of a gas is to determine the influence empirically. This being said, it is also reasonable to develop the salting out relationship for a generalized solution that represents an 'average' working solution and use the empirically obtained parameters for estimation of 'similar' solutions; such would be the case for hydroponic solutions that change over time, but not to a significant enough degree that would dramatically alter the estimation parameters.
Table A1.1: Hoagland solution composition on an elemental basis (except N, which is presented as the two molecular forms available to plants). The values in brackets are a typical concentration in the soil solution found in productive agricultural soils. from: Principles of Plant Nutrition 5th ed., Mengel and Kirkby, 2001.

<table>
<thead>
<tr>
<th>Macronutrient Components</th>
<th>Concentration (mol m$^{-3}$)</th>
<th>Micronutrient Components</th>
<th>Concentration (mmol m$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate</td>
<td>14 (3.100)</td>
<td>Iron</td>
<td>25</td>
</tr>
<tr>
<td>Ammonium</td>
<td>1 (0.048)</td>
<td>Boron</td>
<td>46</td>
</tr>
<tr>
<td>Potassium</td>
<td>6 (0.510)</td>
<td>Manganese</td>
<td>9 (0.002)</td>
</tr>
<tr>
<td>Calcium</td>
<td>4 (1.650)</td>
<td>Zinc</td>
<td>0.8 (0.48)</td>
</tr>
<tr>
<td>Magnesium</td>
<td>2 (0.490)</td>
<td>Copper</td>
<td>0.3</td>
</tr>
<tr>
<td>Phosphorous (phosphate)</td>
<td>1 (0.002)</td>
<td>Molybdenum</td>
<td>0.2</td>
</tr>
<tr>
<td>Sulphur (sulfate)</td>
<td>2 (0.590)</td>
<td>Chloride</td>
<td>18</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration (mmol m$^{-3}$)</th>
<th>Component</th>
<th>Concentration (mmol m$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate</td>
<td>750</td>
<td>Iron</td>
<td>2</td>
</tr>
<tr>
<td>Ammonium</td>
<td>100</td>
<td>Boron</td>
<td>3</td>
</tr>
<tr>
<td>Potassium</td>
<td>250</td>
<td>Manganese</td>
<td>1</td>
</tr>
<tr>
<td>Calcium</td>
<td>250</td>
<td>Zinc</td>
<td>0.5</td>
</tr>
<tr>
<td>Magnesium</td>
<td>100</td>
<td>Copper</td>
<td>0.1</td>
</tr>
<tr>
<td>Phosphorous (phosphate)</td>
<td>0.04 to 25</td>
<td>Molybdenum</td>
<td>0.02</td>
</tr>
<tr>
<td>Sulphur (sulfate)</td>
<td>100</td>
<td>Cobalt</td>
<td>0.04</td>
</tr>
<tr>
<td>Chlorine</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
If the values in Table 2 are summed and converted to mol L\(^{-1}\), then the solution would have a 'component concentration' of 1.68 mmol L\(^{-1}\). This concentration range is well below a molar solution and can therefore be considered a dilute solution with little impact on gas solubility in terms of salting out effects.

There is a lack of literature on the influence of nutrient solution composition on ozone solubility. Given the nature of the research program that I am involved with, this is clearly an area of research opportunity that needs to be taken advantage of.

**LOSS OF AQUEOUS OZONE DUE TO SPRAYING**

In many of the envisaged agricultural applications of O\(_3\)\(_\text{aq}\), the solution will be applied as a spray. This is a potential area of concern, as the general trend under most spraying conditions will be for ozone to come out of solution. There are several contributing and regulating factors that will dictate the amounts and rates of loss from solution upon spraying. The key parameters include: 1) operating pressure; 2) nozzle type; 3) drop size; 4) \([O_3\text{aq}]\) and \([O_3\text{g}]\); 5) temperature; and 6) reactions. There are numerous other minor contributors to the overall loss of ozone from solution upon spraying, but they will not be considered in this discussion.

The bulk loss of ozone from solution during a spray application to a crop has been examined by Graham et al. (2009). Fugi and Fugiwara (2002) also examined the loss of ozone upon spray application in a lab setting. Both of these studies only looked at the bulk loss from solution. The concentration before and after was measured and a percentage loss reported. In both of these studies, the loss rates were significant, ranging from 65-75% (Graham et al. 2009) to 90% (Fugi and Fugiwara, 2002). These studies cannot be directly compared as the number of parameters differing between the procedures precludes any useful comparison. These studies do highlight the wide variability in ozone loss from solution as a function of numerous controlling parameters, many of which are unique to each target application (e.g. Pressure, air exposure distance, nozzle type, etc).

The following sample calculation is presented to give an idea of the losses that may be expected upon atomization of an ozone solution. I will point out now that in order to simplify the calculations, numerous assumptions have been made that, in all likelihood, have lead to a significant over estimation of the final gas phase concentrations in the air parcel.

**Example:**

Assumptions:
1) The system is laminar
2) All drops are the same size
3) Gas phase resistance is negligible
4) No chemical reactions (no ozone loss)
5) The liquid film interface concentration is 90% that of the bulk solution
Fig. A1.2: Two-film mass transfer model in a water droplet system. The layers depicted in the model presented in Fig. 1 are present in concentric spheres. To account for the spherical geometry, the surface area : volume ratio for the drop is incorporated into the specific mass transfer rate. The standard concentration gradient is shown in red; however, for the calculations presented the gas phase is assumed to be uniform (i.e. single film layer).
Known or predetermined:
1) Drop diameter is 2.5 mm
2) Ozone diffusivity (D) is $1.76 \times 10^{-9} \text{ m}^2 \text{s}^{-1}$
3) $\delta_L$ (depth of liquid film layer) is 200 um
4) Concentration in the bulk drop is 1.0 mg L$^{-1}$

Mass Transfer Flux (N) out of liquid phase (equals the flux into the gas phase)

$$N = k_L(c_L - c_{Li})$$  \hspace{1cm} \text{Equation 3.1}

where:
- $k_L$ is the mass transfer coefficient for the liquid phase
- $c_L$ is the concentration in the bulk liquid
- $c_{Li}$ is the concentration at the interface

Specific Mass Transfer rate (m) takes into account the spherical geometry.

$$m = k_L a(c_L - c_{Li})$$  \hspace{1cm} \text{Equation 3.2}

$$a = A/V_L$$  \hspace{1cm} \text{Equation 3.3}

where:
- $a$ is the specific surface area ratio (transfer surface per unit volume)
- $A$ is the surface area of the sphere
- $V_L$ is the volume of the sphere

Mass Transfer Coefficient ($k_L$)

$$k_L = D/\delta_L$$  \hspace{1cm} \text{Equation 3.4}

Solving equation 3.3:

$$a = (\pi d^2)/((\pi d^3)/6) = (\pi(0.0025\text{m}^3))/((\pi(0.0025\text{m}^3))/6)$$

$$= 1.9634 \times 10^{-5}\text{m}^2 / 8.18 \times 10^{-9}\text{m}^3$$

$$= 2.4 \times 10^3 \text{m}^{-1}$$

Solve for equation 3.4:

---

26 Typically this value is estimated and included in $k_L$ as it is difficult without sophisticated equipment to measure the specific area of a swarm of irregular drops in a stream.

27 This is a simplified version that makes specific assumptions regarding the concentration gradient in the liquid film layer and relates the coefficient specifically to the film depth.
\[ k_L = \frac{D}{\delta_L} = 1.76 \times 10^9 \text{ m}^2\text{s}^{-1} / 2.0 \times 10^{-4} \text{m} \]

\[ = 8.8 \times 10^{-6} \text{ m s}^{-1} \]

Solve for Equation 3.2:

\[ m = k_L a(c_L - c_{Li}) = 8.8 \times 10^{-6} \text{ m s}^{-1} \times 2.4 \times 10^3 \text{ m}^{-1} \times (1.0 \text{ mg L}^{-1} - 0.9 \text{ mg L}^{-1}) \]

\[ = 2.1 \times 10^2 \text{ s}^{-1} (0.1 \text{ mg L}^{-1}) \]

\[ = 2.1 \times 10^3 \text{ mg L}^{-1} \text{s}^{-1} \]

Based on this mass transfer rate, all the ozone would come out of the drop within a second of leaving the nozzle. The assumptions made clearly are not realistic, and many other factors need to be considered when attempting to calculate the loss from solution. This being said, the studies by Graham et al. (2009) and Fugi and Fugiwara (2002) did show dramatic losses upon spraying. In these trials, the actual 'air time' of the spray was 1 second or less based on the distances indicated in the papers. Even with this short air exposure (drops captured upon landing) the losses were high, indicating that although the presented example is an over exaggeration of the mass transfer rate, it is likely that it represents a reasonable first approximation.

The many contributing factors to an accurate mass transfer model are typically empirical in nature (need to be determined empirically for the application of interest). Over the course of the project, many of these parameters will be established for irrigation solutions and a better estimate of mass transfer will be available.

4. SUMMARY

Characterizing the solubility of a reactive gas such as ozone is a difficult task. In all but the most pristine systems, the number of influencing factors is significant. During this discussion I have attempted to address several of the key factors that are relevant to hydroponic and irrigation solutions. In actual applications, the solubility and stability of ozone in solution will be governed by the characteristics of the source water. Every system will therefore be different and will require characterization prior to establishing a treatment system. This uncertainty is perhaps the most attractive aspect of this work from a research point of view, as it provides a nearly limitless source of research questions.
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; Runia, 1994; Stewart-Wade, 2011), 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

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28 The material presented in this chapter is taken in whole or in part from a short communication of the same title appearing in the Journal of Horticulture and Forestry. 3(2):58-62. (2011). The format of that original manuscript is preserved here, however, it is atypical.
performance (Drew 1997; Zheng 2007). Growers incorporating ozone into their irrigation management strategy typically allow the ozone to dissipate or actively remove it prior to distribution to the 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 mineral wool 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 mineral wool hydroponic substrate suppressed productivity (as measured by leaf area and dry matter accumulation); and 2) determine if O$_3$(aq) applied directly to a mineral wool growth substrate can reduce the incidence of *P. aphanidermatum*.

**RESULTS & DISCUSSION**

Although these studies are limited in scope, there was clear indication that aqueous ozone could be applied directly to the surface of mineral wool growth substrate in both tomato and cucumber hydroponic culture without adversely influencing growth (Fig. A2.1). 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 (Fig. A2.2).
Fig. A2.1: Leaf area (LA) and shoot dry weight (SDW) response of tomato and cucumber to direct applications of aqueous ozone to the mineral wool (Grodan Delta-10 Gro-blocks 10cm x 10cm x 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 L aliquot from one of five O$_3$(aq) solutions (0, 5, 10 15, 20 mg·L$^{-1}$). The solutions were poured over the mineral wool cube at an average rate of 1 L·min$^{-1}$. Plants were grown for an additional 12 d 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 L aliquot from one of four O$_3$(aq) solutions (0, 5, 10 15 mg·L$^{-1}$). The solutions were poured over the mineral wool cube at an average rate of 1 L·min$^{-1}$. Plants were grown for an additional 10 d 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 L O$_3$(aq) solution (0, 2, 4, 6 mg·L$^{-1}$). Treatments commenced when the plants were six weeks old and continued for 6 days, after which the plants were grown for an additional 7 d before being destructively analysed: A-F) Columns falling under the same horizontal line are not statistically different at P<0.05; error bars are +/- SE 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™ 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.
A

B

C

D

E

F

Tomato 1x application

Cucumber 1x application

Tomato 2x daily for 6-days

Leaf Area (cm²)

Shoot Dry Weight (g)

Leaf Area (cm²)

Shoot Dry Weight (g)

Leaf Area (cm²)

Shoot Dry Weight (g)

Leaf Area (cm²)

Shoot Dry Weight (g)

Leaf Area (cm²)

Shoot Dry Weight (g)

Leaf Area (cm²)

Shoot Dry Weight (g)
In the first two studies 2 L aliquots of solutions containing high O$_3$(aq) concentrations (5, 10, 15, 20 mg L$^{-1}$) were applied in a single dose to the root zones of tomato and cucumber plants. The results (Fig. A2.1 A-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$^{-1}$) or no ozone remained in the solution after passage through the mineral wool 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 microorganisms. Repeated applications may overwhelm this buffer capacity. In a third study the concentrations applied were reduced to 0, 2, 4, 6 mg L$^{-1}$ O$_3$(aq) but were applied twice daily for six days. Once again the results (Fig. A2.1 E-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 a week for three weeks at 1.5 mg L$^{-1}$, 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 *Pythium aphanidermatum* and subjected to a series of ozone treatments. The results are summarized in Fig. A2.2.
Fig. A2.2: Response of cucumber plants grown in mineral wool growth media (Grodan Delta-10 Groblocks 10 cm x 10cm x 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$^{-1}$ 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 mineral wool substrate twice daily (1 L min$^{-1}$; applied at 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$^{-1}$ 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 mineral wool substrate twice daily (1 L min$^{-1}$; applied at 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 with the same letter appearing above it as other columns in the group are not significantly different at P<0.05; error bars are +/- SE 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™ 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 and were 42 d old at harvest. *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 – disease symptoms not detected.
Although the application of O\textsubscript{3(aq)} clearly reduced root disease in both studies (Fig. A2.2 A and D), it did not necessarily result in a maintenance of productivity under intense disease pressure (Fig. A2.2 E-F). Given the limited scope and the failure for the studies to corroborate one another in terms of productivity maintenance (Fig. A2.2 B-C, E-F), only limited inferences can be made on the efficacy of O\textsubscript{3(aq)} to prevent production loss due to disease. The root disease was clearly reduced in both studies (Fig. A2.2 A and D), which suggested that ozone was 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\textsubscript{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\textsubscript{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 Fig. A2.2 A-C, but rather that the complexities of the systems should not be underestimated.

The results presented justify a more thorough evaluation of the direct application of O\textsubscript{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 are different than that of the gas phase. This knowledge opens the door to a range of horticultural O\textsubscript{3(aq)} applications related [primarily] to irrigation system maintenance and pest management.