OZONE (O_3) EFFICACY ON REDUCTION OF *PHYTOPHTHORA CAPSICI* IN RECIRCULATED HORTICULTURAL IRRIGATION WATER

A Dissertation

by

GARRY VERNON McDONALD

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2007

Major Subject: Horticulture

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Approved by:

Co-Chairs of Committee,	Michael A. Arnold
	Don C. Wilkerson
Committee Members,	Bruce J. Lesikar
	W. Todd Watson
Head of Department,	Tim D. Davis

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ABSTRACT

Ozone (O_3) Efficacy on Reduction of *Phytophthora capsici* in Recirculated Horticultural Irrigation Water. (May 2007)

Garry Vernon McDonald, B.S., Texas A&M University;

M.S. Texas A&M University

Co-Chairs of Advisory Committee: Dr. Michael A. Arnold
Dr. Don C. Wilkerson

Microorganisms that cause plant disease have been isolated in recirculated irrigation water and increase the risks of disease incidence in horticultural operations. Ozone is an effective oxidizer used to disinfect drinking water supplies and treat industrial wastewater. The objective of this research was to investigate using ozone gas as part of a strategy to reduce the incidence of *Phytophthora* deBary in recirculated irrigation water. An isolate of *Phytophthora capsici* Leonian was cultured to induce sporulation. Spore dilutions were placed in aliquots of reverse osmosis water and bubbled with ozone gas (O_3) to concentrations of 0 to 1.5 mg·L^{-1}. Ozonated samples were plated and observed for colony forming units.

Increasing ozone concentrations reduced the number of colony forming units to 0 at 1.5 mg· L^{-1} 0_3. Turbidity effects on efficacy on *Phytophthora capsici* were tested using bentonite clay at 0 to 2.0 nephelometric turbidity units and ozone concentrations of 0 to 1.5 mg· L^{-1}. Increasing bentonite did not affect the efficacy of increasing ozone concentrations on reducing colony formation to 0 at 1.5 mg·L^{-1} O_3. Bioassays using *Phytophthora capsici* on *Capsicum annuum* L. seedlings confirmed apparent pathogenicity. Reverse osmosis water, containing a soluble fertilizer at 0 to 300 mg· L^{-1} N, was ozonated to concentrations of 0 to 1.5 mg·L^{-1} O_3 and used to irrigate *Chrysanthemum* x *morifolium* T. de Romatuelle. Increasing ozone concentrations did not interact with increasing fertilizer levels to affect the final growth parameters. Chrysanthemum exposed to ozone gas concentrations of 0.5 to 1.5 mg·L^{-1} showed symptomatic ozone damage. Complete soluble fertilizer solutions with micronutrients

were ozonated from 0 to 1.5 mg·L^{-1} O_3 and analysed for nutrient content. Increasing ozone levels did not interact with fertilizers to affect macronutrients. Increasing ozone interacted with iron at a high fertilizer level. Ozone did not affect the efficacy of paclobutralzol in controlling growth in *Viola* x *wittrockiana*. Ozone was effective in controlling *Phytophthora capsici* in recirculated irrigation water with minimum impact on plant growth. Adjustments in fertility regiemes may be needed to counteract the oxidizing affect of ozone on micronutrients.

DEDICATION

To my teachers
All knowledge is truly cumulative

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CHAPTER I
INTRODUCTION

Consumers demand high quality containerized bedding plants, shrubs, trees, and other ornamental plants produced by the horticultural industry. This demand for quality exhorts pressure on horticultural producers to grow aesthetically pleasing plants free from pest and blemishes. Production of such plants results in the luxurious consumption of water and agricultural chemicals with concomitant potential for nutrient and pesticide runoff (Yelanich and Biernbaum, 1990). Plant growth regulators (PGR) are another class of agricultural chemicals of recent concern. There is the possibility of PGR residue runoff in recycled irrigation water and its implications in unwanted growth retardation at low levels (Adriansen, 1997; Arnold and McDonald, 2001; Million et al., 1999). In addition, ground and surface water usage and rights issues are becoming increasingly important in many areas of the country (Wilkerson and Arnold, 1994; Wilkerson, 1995). Having less water of lower quality is a fact the industry may face. To reduce water consumption and mitigate pollution runoff, horticultural operations may capture and recycle irrigation runoff. The Water Pollution Control Act sets the standard for clean water and prescribes point-discharge requirements for federal, state, and regional water districts (U.S. Congress, 1972). Because of these standards, specific concerns to the horticulture production managers include, irrigation and rainfall runoff, capture, recycling, deactivating organic and inorganic agricultural chemicals and water disinfection to control the spread of plant pathogens. The conundrum the horticulture industry faces is to maximize water use efficiency, minimize water contamination, and reduce the volume of chemicals applied to crops while maintaining a high quality product. An area of major concern for many producers is the possibility of large-scale plant pathogen infestations due to contaminated recycled water (Thompson and Allen, 1974).

This dissertation follows the style of the Journal of the American Society for Horticultural Sciences.

As alluded to, many operations capture and re-use vast amounts of water with some peak usage rates reported as high as 190-750 x 10^3 L ·hr^{-1} that is re-distributed over large areas (MacDonald et al., 1994). The potential for irrigation water to become infested with plant disease causing organisms and then becoming widely dispersed throughout the growing facility is great (Jenkins and Averre, 1983; Runia, 1995; van Os, 1999).

The incidence of plant pathogens being spread by contaminated irrigation water is documented (Shokes and McCarter, 1979; Thompson and Allen, 1974; Whiteside and Oswalt, 1973). Specific plant pathogenic organisms of concern include but are not limited to *Rhizoctonia* DC, *Phytophthora* deBary, *Pythium* Pringsh, and *Fusarium* Schlechtendahl species (Barnes, 2004; Reeser, 1998). Bacteria are usually less of a problem, but the possibility exists for the spread of *Erwinia* (Townsend) Holland and *Pseudomonas* E. F. Smith (Joiner, 1981). Organisms in infected tissue reproduce and form either asexual or sexual propagules that wash off infected plants into the irrigation runoff (Joiner, 1981). Irrigation water that runs off from these diseased plants may be captured and recycled (Reeser, 1998). Captured runoff water may be enriched with nutrients from fertility programs and with organic or inorganic particulate matter that may further facilitate microbial infestations through enriched environments, or by interfering with water treatment systems such as filtration (MacDonald et al., 1994). *Phytophthora*, in particular, has been reported as a major contaminant of recirculated nursery irrigation water (MacDonald et al., 1994)

A major component in overcoming such potential disease problems is to disinfect the captured runoff water. Traditional methods for treating water in municipal drinking supplies and industrial waste water present challenges to the horticultural industry. The most common method of treating any water supply (either potable or industrial wastewater) including nursery irrigation water is the injection of chlorine or bromine, as chlorine or bromine gas, into the water stream (Ferraro and Brenner, 1997). Treatment concentrations vary widely depending on types of influent or effluent being treated. Normal domestic wastewater effluents require a range from 1 to 3 mg·L^{-1} residual free chlorine to meet most state and federal standards (De Hayr et al., 1994; Grasso, 1996). Water sources high in organic and inorganic contaminants may require as much as 25 to

30 $mg \cdot L^{-1}$ chlorine to achieve the required 1 to 3 $mg \cdot L^{-1}$ residual chlorine due to chemical binding (De Hayr et al., 1994). In addition, 1 $mg \cdot L^{-1}$ of free chlorine (in the form of $HOCl^{-2}$) has been shown to inactivate many water-borne viruses (Rubin, 1975). These recommendations are based on chlorine's efficacy at keeping coliform bacteria at or below established threshold levels. The major factor for the use of chlorination in water treatment in horticultural operations is low to moderate levels (>7 $mg \cdot L^{-1}$) of chlorine may be lethal or phytotoxic to many plant species (Bugbee, 1987; Ferraro and Brenner, 1997). In addition to negative plant growth responses, chlorine can be very corrosive to equipment, may form undesirable secondary organic by-products such as trihalomethane, and can be an explosive and health hazard (Grasso, 1996).

An alternative method of treating recycled water is the use of radiant energy in the form of ultraviolet radiation (UV) excimer lamps emitting radiation at 172 and 222 nm (Ramsay et. al, 2000). The use of ultraviolet radiation in a disinfection procedure works by inducing photobiochemical changes within a microorganism. Two criteria must be met in order for UV light to be effective; namely, the radiation must be of sufficient energy to alter chemical bonds, and the radiation must be adsorbed by the organism (Grasso, 1996). The ability to deliver radiation from the UV generating source to the target organism is crucial to the performance of UV disinfection systems. The major problem in UV disinfecting systems in horticultural production facilities is dissolved and fine particulate matter causing turbidity in the water to be treated. These materials may impede or absorb UV radiation transmission through the liquid being treated. Limitations on radiation delivery can also be caused by the deposition of insoluble materials, such as various mineral salts, on the surface of the quartz jackets that typically surround and house the UV source lamps (Grasso, 1996), resulting in a process known as fouling. Hard water or water high in iron oxides (Fe^{3+}) may also result in fouling of the lamp surfaces. Another disadvantage to using UV radiation is a phenomenon known as photoreactivation and dark repair in which microorganisms have evolved biological systems to repair damage by sub-lethal exposure to disinfectants such as UV light. This phenomenon can be overcome by treating the water with successive UV light exposures followed by a dark period. Such systems are costly to build, maintain, and operate and

are not usually feasible for most horticultural operations. Other water treatment strategies include heat, oxidizing chemicals, and membrane filters (Ehret et al., 2001).

CHAPTER II
OZONE AND WATER TREATMENT: AN OVERVIEW

Ozone has been used to treat drinking water, especially in France, since the early 1900's (Brink et al.,1991; Rideal, 1920). Because of high equipment cost and low ozone generation efficiency, ozone disinfection has not been widely used in the United States until recently with the development of improved ozone gas generators and reduced equipment cost (Martin, 1991). Ozone, when used as a disinfectant in dosages of 3 to 10 $mg \cdot L^{-1}$, is highly effective in inactivating common forms of bacteria, bacterial spores, fungi, and harmful viruses in wastewater effluent. Ozone, under higher treatment concentrations and under catalytic conditions, can also chemically oxidize organic waste materials and deactivate certain pesticides, herbicides, and flocculate out suspended solids (Rice and Browning, 1981; Rivas et. al., 2001; Runia, 1994). As a disinfectant, ozone has an advantage over other compounds in that ozone is short-lived (half-life = 20 min.) in an aqueous solution and breaks down into elemental oxygen (O_2).

Some potential problems do occur when using ozone. Off gassing from excessive dosage or leakage can be a major concern in areas with poor air quality and stringent gas or pollution emission restrictions. De-ozonation of excess ozone generation may be required at some sites. A more immediate concern to the nursery/floral industry is the effect of ozone on plant growth due to either direct tissue damage from high residual ozone levels or indirectly from interaction with fertility regimes, mainly fertilizers injected into the irrigation stream (fertigation). Health issues will be discussed separately.

Ozone is an inherently unstable gas that can not be stored and therefore must be produced or generated at the point of use. In a gaseous state, ozone has a half-life (point in time where one-half of the original ozone concentration is reduced to elemental oxygen O_2) of about 12 h in the ambient atmosphere. However, in an aqueous solution ozone's half-life is much shorter at around 20 min. in distilled water. Pure water has a low concentration of ozone-demanding constituents with a corresponding longer residual time. In a comparison between various water sources and the half-life of ozone under identical temperatures, Rosenthal (1974), found a half-life as short as 10 min. in filtered lake water and greater than 1 h in double distilled water. In practical terms, the longer

the half-life or residual time, the greater the ability to inactivate any contaminants in that water source. Conversely, turbid or waters otherwise high in contaminants will require a higher ozone concentration over a longer time period to achieve effective inactivation of those contaminants. Acid conditions (pH 5 to 7) facilitate better microbial or contaminant inactivation because of the higher ozone stability at those pH ranges (Farooq et al., 1977). The water's constituents have a major effect on ozone's disinfection ability.

As mentioned previously, ozone must be generated at the point of usage. Traditionally, ozone was generated by a corona discharge method. This method was previously preferred because it generated the greatest concentration of ozone gas per electrical unit input (Rice et al., 1986). In this procedure, an ambient air source or pure oxygen gas source is heated or otherwise chemically dried (dew point of -40 °C) to remove moisture or humidity from the gas. The gas is passed between two electrically charged plates separated by a ceramic dielectric substance across a narrow discharge gap. As the gas passes through the discharge gap, part of the oxygen in air or supplied oxygen is converted to ozone. This synthesis is a equilibrium reaction with the reverse reaction increasing at temperatures above 35° C (Rice et al., 1986). Because of the amount of electrical current needed to effect the reaction, a large amount of waste heat is generated that must be dissipated. The corona discharge method is capable of generating ozone concentrations of 12 to 38 mg $\cdot$ L^{-1} when ambient air is used with pure oxygen generating concentrations of 103 to 155 mg $\cdot$ L^{-1} (Flusche, 2006). This system of ozone generation requires large amounts of electrical power to operate both the corona discharge plates and the necessary cooling plant to maintain the equipment and prevent the reverse reaction from ozone to oxygen. In addition, significant inputs are required to pre-treat ambient air streams and the expense and danger associated with liquid oxygen supplies and storage. Ultraviolet (UV) light ozone generators also exists. The generators have limited application because of the minimum ozone output (< 1.2 mg $\cdot$ L^{-1}) (Flusche, 2006).

Recent technological innovations in ozone generation offer greater opportunities for a wider use and application of ozone in water treatment schemes. Electrochemical ozone generators have been developed using electrochemical cell stacks. The basic chemical process is the electrolysis of water. A direct current (DC) is applied across the anode and cathode sources positioned on either side of a Nafion 117 (DuPont) proton-exchange

membrane (PEM), a fluoropolymer highly resistant to chemical degradation (Flusche, 2006). When water is fed to the anode (+) electrode, which is coated with lead dioxide, two oxidation reactions take place. Reaction 1 is an oxygen evolution reaction. Reaction 2 is an ozone formation reaction summarized as follows:

Reaction 1. $2H_2O + 4\ e^- \rightarrow O_2 + 4\ H^+$ $\quad E^\circ = 1.23$ V

Reaction 2. $3H_2O + 6\ e^- \rightarrow O_3 + 6\ H^+$ $\quad E^\circ = 1.51$ V

At anodic potentials greater than 1.23 V, the lead dioxide coating on the electrode can catalyze the ozone formation reaction (reaction 2 above) while reducing the oxygen generating reaction (reaction 1 above). The ozone and oxygen generated partitions between the liquid and gas phases as they are produced. Hydrogen protons move to the cathode electrode via an external circuit where they react with electrons to produce hydrogen gas which may be vented or combined with oxygen via a catalyst to produce water vapor which is also vented. The reaction is:

$2H^+ + 2\ e^- \rightarrow H_2$ $\quad E\circ = 0.0$ V

The proton-exchange membrane is a solid electrolyte which serves to conduct protons between the two electrodes. The solid electrolyte has several advantages over a liquid electrolyte. The potential leakage of corrosive chemicals is eliminated. Additionally, the solid proton-exchange membrane separates the two electrodes and the Nafion anode interface by providing a favorable environment which allows for greater electrochemical ozone formation. The Nafion membrane, as mentioned, is highly resistant to chemical attack by ozone. A final cathode reaction involves reducing oxygen from ambient air and protons (H^+) flowing from the anode to the cathode via the proton-exchange membrane to produce water and is represented by the reaction:

$O_2 + 4H^+ + 4\ e^- \rightarrow 2\ H_2O$ $\quad E^\circ = 1.23$ V

Individual electrochemical cells can be "stacked" to form multi-cell units to scale up production to a desired output. Electrochemical generation can produce an ozone output of 12 to 16 percent by weight and has the advantage of using good quality water (reverse osmosis or better) as a feed source thereby eliminating the need for expensive air drying equipment or liquid oxygen (Flusche, 2006).

Ozone along with hydroxyl radicals are considered two of the strongest chemical oxidants (Gottschalk et al., 2000). Ozone can act directly on an microorganism and other substances or indirectly by producing secondary hydroxyl radicals that then react with a targeted organism or substance (Gottschalk et al., 2000). These two reaction types, indirect and direct, follow different pathways and have different oxidation products and are controlled by different reaction kinetics; however, they usually interact. Many substances react instantaneously with ozone. Metals such as iron, manganese, many organic complexes with heavy metals, and phenols react very quickly. In addition, many types of algae and inorganic compounds such as cyanide, sulfide, and nitrite also react quickly. Conversely, other types of organic material reacts more slowly with ozone. Detergents, pesticides, organo-nitrogen, organic acids, and some algae react much slower with ozone (Rice et al., 1986).

The indirect reaction involves the decay of ozone into radicals and is accelerated by initiators such as OH^- which in turn form secondary hydroxyl radicals (OH°). Other initiators include peroxides (H_2O_2, HO_2^-) and Ferrous Iron (Fe^{2+}). These radicals, acting as oxidants, react instantaneously with solutes and are nonselective. The actual radical pathway is complex and influenced by many factors. The pathway is divided into three different stages namely the initiation step, the radical chain reaction, and a final termination step (Gottschalk et al., 2000).

An initial reaction between ozone and hydroxide ions leads to the formation of one superoxide anion radical and one hydroperoxyl radical and summarized by the equation:

$$O_3 + OH^- \rightarrow O2^{\circ -} + HO_2^\circ \qquad k = 70\ M^{-1}\ s^{-1}$$

The hydroxyl radical is in an acid-base equilibrium:

$$HO_2^\circ \leftrightarrow O_2^{\circ -} + H^+ \qquad pK = 4.8$$

The products of this initial reaction with additional ozone are used in a chain reaction phase to produce hydroxyl radicals (OH°) and summarized as follows:

$$O_3 + O_2^\circ\text{-} \rightarrow O_3^{\circ -} + O_2 \qquad k = 1.6\ 10^9\ M^{-1}\ s^{-1}$$

$$HO_3^\circ \leftrightarrow O_2^{\circ -} + H^+ \qquad pK = 6.2$$

$$HO_3^\circ \rightarrow OH^\circ + O_2 \qquad k = 1.1\ 10^8\ M^{-1}\ s^{-1}$$

The hydroxyl radical (OH°) reacts with ozone to produce:

$$OH^\circ + O_3 \rightarrow HO_4^\circ \qquad k = 2.0\ 10^9\ M^{-1}\ s^{-1}$$

$HO_4^{\circ} \rightarrow O_2 + HO_2^{\circ}$ $\quad k = 2.8\ 104\ M^{-1}\ s^{-1}$

The decay of HO_4° into oxygen (O_2) and the hydroperoxide radical (HO_2°) allows for the reaction to start again leading to a chain reaction producing hydroxyl radicals (OH°) which act as the main oxidizing element. Materials which can convert the hydroxyl radical into superoxide radicals ($O_2^{\circ-}$, HO_2°) promote the chain reaction and are known as promoters. Organic molecules (R) can act as promoters which have functional groups that can react with the hydroxyl radicals to form organic radicals (R°) represented by:

$H_2R + OH^{\circ} \rightarrow HR^{\circ} + H_2O$

When oxygen is present, organic peroxy radicals (ROO°) can form and further react with the superoxide radicals ($O_2^{\circ-}$, HO_2°) to form further hydroxyl radicals which then can enter the chain reaction as represented by:

$HR^{\circ} + O_2 \rightarrow HRO_2^{\circ}$

$HRO_2^{\circ} \rightarrow R + HO_2^{\circ}$

$HRO_2^{\circ} \rightarrow RO + OH^{\circ}$

The decay of ozone and the subsequent chain reaction produces fast reacting and non-selective hydroxyl radicals (OH°). These hydroxyl radicals are electrophilic and react at a molecule's bond position that has the highest electron density (Gottschalk et al., 2000).

Certain organic and inorganic molecules react with the hydroxyl radical (OH°) to form secondary radicals that do not produce superoxide radicals ($O_2^{\circ-}$, HO_2°) and therefore induce an inhibitory effect. These inhibitors, also known as scavengers, can terminate the chain reaction and inhibit ozone decay and thus hydroxyl radical formation. Examples of scavengers include bicarbonate and carbonate, phosphates, humic or other organic acids, and *tert*-butyl alcohol. In the overall multi-step indirect reaction, three ozone molecules decay to produce two hydroxyl radicals as follows:

$3\ O_3 + OH^- + H^+ \rightarrow 2\ OH^{\circ} + H_2O$

In the direct reaction, ozone reacts directly at unsaturated carbon bonds (C=C) due to the dipolar structure and splits the bonds as summarized by the reaction:

$$\begin{array}{l} \qquad\qquad\qquad\quad O \\ \qquad\qquad\quad | \qquad\quad \| \\ -C=C- + O_3 \rightarrow -C=O \text{ or } -C-OH \end{array}$$

Ozone will react quicker with substances that have electron supplying substituents (hydroxyl groups) (Gottschalk et al., 2000). The reaction will be slower if no such substituent components are available. Ionized organic compounds react faster with ozone than neutral compounds. The direct ozone reaction becomes important if the radical producing reactions become inhibited. In aqueous solutions, if initiators are absent to form the chain reaction or scavengers are present with terminate the chain reaction, the direct reaction dominates. Under acidic conditions (pH < 4) the direct reaction predominates while in basic (pH > 10) conditions the indirect reactions predominate. In neutral (pH ≈ 7) aqueous conditions, both direct and indirect reactions contribute to ozone decay and subsequent oxidation.

Toxicology examines the adverse affects of substances on living organisms. Two terms are normally used when describing toxicological events. Acute toxicology is a fast harmful effect after only a short exposure time or an exposure in limited amounts. Chronic toxicology concerns the harmful effects caused by a substance over a prolonged time period (Gottschalk et al., 2000). When considering toxicity, terms used include lethal dose (LD) or lethal concentration (LC). LC_{50} is the concentration where 50% of a targeted population dies. The effective dose or concentration (ED or EC) is defined analogously where EC_{50} is used to describe adverse effects in 50% of the test organisms within the prescribed exposure period (Gottschalk et al., 2000).

Ozone is a highly toxic, oxidizing gas. Routes of entry into humans include inhalation, skin penetration, and eye contact. The most common mode of ozone exposure is inhalation of ozone gas. However, ozone effects differentiate when application uses vary such as ozone in gas, ozone in liquid, and ozone by-products (Gottschalk et al., 2000).

Ozone exposure concentrations of a few tenths of 1 mg · L^{-1} (1 mg · L^{-1} = 2 mg · m^{-3} at 20° C and 101.3 kPa) can cause occasional discomfort in the form of headaches, dry throat and mucous membranes, and irritation of the nose (Gottschalk et al., 2000). Ozone has an olfactory (odor detected) threshold of about 0.02 mg · L^{-1} but also has a desensitizing effect over time (Gottschalk et al., 2000). Exposure to high concentrations or prolonged exposure can have serious health manifestations. Toxic symptomology includes lung edema, frontal headaches, substernal pressure, constriction or oppression,

acid taste in the mouth, and anorexia. Severe acute exposure to ozone can produce dyspnea (difficult or labored breathing), cough, choking sensation, tachycardia (rapid heart rate), vertigo, low blood pressure, cramping chest pain, and generalized body pain. An estimated 50 mg · L^{-1} exposure for 30 minutes is considered fatal (Gottschalk et al., 2000).

Chronic exposure symptomology is similar to acute exposure with pulmonary lung function decreasing depending on ozone concentration and duration. Asthma, allergies, and other respiratory disorders have been linked to chronic ozone exposure. *In vivo* studies indicate direct and indirect genetic damage with possible tumorgenic effects. Prolonged exposure is suspected of having carcinogenic potential (Gottschalk et al., 2000).

Direct ozone contact to skin tissue can cause irritation and high concentrations in liquid can cause skin burn and frostbite. Eye irritation can occur at or above levels of 0.1 mg · L^{-1} (Gottschalk et al., 2000).

The American Conference of Governmental Industrial Hygienist (ACGIH) sets exposure limits for work involving ozone gas. Immediately Dangerous to Life or Health (IDLH) level for ozone is 5 mg · L^{-1}. Threshold value limits (TVL) for ozone are 0.05, 0.08, and 0.10 mg · L^{-1} for heavy, moderate, and light work conditions (Gottschalk et al., 2000). For safety reasons, an ambient air ozone monitor (0 to 1 mg · L^{-1} range) should always be present when ozone is in use. Appropriate shut down safety procedures should also be in place (Gottschalk et al., 2000).

No health hazard data are available and no limits for the workplace exist on using ozone in liquids (Gottschalk et al., 2000). Ozonated water in high concentrations can lead to eye and skin irritations. Most of the toxic effects associated with ozone in liquids are related to off-gassing (ozone evolution out of solution). For this reason, ozonated liquids at high concentrations should be used in closed piping or containers (Gottschalk et al., 2000). Langlais et al. (1991) investigated the toxic affects of ozonated water on fish species and found a LD_{50} ranging from 0.0093 mg · L^{-1} for 96 hour exposure in Rainbow Trout *Oncorhynchus mykiss* Walbaum to 0.38 mg · L^{-1} for 24 hour exposure in White Perch *Morone Americana* Gmelin.

CHAPTER III
PHYTOPHTHORA: TAXONOMY AND EPIDEMIOLOGY

Overview

Phytophthora deBary microorganisms account for some of the most destructive and economically significant plant disease epidemics on record (Zentmyer, 1983). The most poignant example is late blight of potato (*Solanum tuberosum* L.) caused by *Phytophthora infestans* (Mont.) deBary which triggered the great famine of 1845-1849 in Ireland (Bourke, 1991; Gregory, 1983). This one epidemic caused massive starvation in which over one million people died and the immediate emigration to North America of another million Irish people; fundamentally altering a whole culture and society. This epidemic occurred just before the advent of modern germ theory and it has been stated that the potato famine triggered the development of plant pathology as a science (Erwin and Ribeiro, 1996). Currently, a pathogenic organism that has caused much concern in the United States and Europe is *Phytophthora ramorum* Werres, DeCock, and Man in't Veld which is the cause of Sudden Oak Death (SOD) in the western United States and leaf blight and canker on many ornamental species including rhododendron (*Rhododendron* L.) and camellia (*Camellia* L.) in the southeastern United States and in Europe. First noticed in 1993 in Germany and Belgium on rhododendron and viburnum (*Viburnum* L.), *P. ramorum* was not described until 2000 and is of an unknown origin (Werres et al., 2001). This organism is of concern because it is not host specific, but infects a wide number of both woodland and landscape species (Tjosvold et al., 2006). The list of susceptible species continues to grow as multiple governmental and scientific agencies monitor this organism's spread (U.S. Department of Agriculture, 2005). In 2003-2004, over two million containerized plants potentially infested with *P. ramorum* were shipped from the west coast of the United States to nurseries and garden centers in 49 of the 50 states (U.S.Department of Agriculture, 2005). California and Oregon have reported infestations in native forests (Rizzo et al., 2002). *Phytophthora ramorum* can potentially adversely affect forest ecosystems through increased fire and safety hazards from dead trees, in addition to negative economic impacts such as lost lumber revenues and reduced property values. The affect on urban landscapes will also be great through

loss of mature trees and shrubs and the subsequent cost of tree removal and replacement. The economic impact on the $13 billion a year horticultural industry will be great due to quarantine restrictions, stop sell orders, and the destruction of infected nursery stock (U.S. Department of Agriculture, 2005). The above are but a couple of examples of the immense impact on mankind from diseases caused by *Phytophthora*.

Given the fact that species of *Phytophthora* cause worldwide epidemics, what characteristics and physiological aspects account for the virulent nature of these organisms? *Phytophthora* causes root and collar rots, leaf spots, twig, and seedling blights on a wide range of plants from herbaceous annuals to forest trees. The genus *Phytophthora*, along with the genus *Pythium* Springsheim, are commonly referred to as "water molds" because both are favored by free water in soil and on plant foliage or other moist humid environments. Pathogenic species of *Phytophthora* are reported in natural streams, ponds, and in recirculated nursery irrigation effluents (MacDonald et al., 1994) While many species of *Pythium* are plant pathogens, many are saprophytes or even parasitic on *Phytophthora* (Erwin and Ribeiro, 1996). Most species of *Phytophthora* are generally considered to be plant pathogens. A moist wet condition is an important environmental component for disease development caused by species of *Phytophthora*; however, *P. ramorum* is unique in that it lacks a strict association with wet soils or free water with symptomatic trees being found on dry hillside sites (Rizzo et al., 2002). Cankers can appear on trunks instead of the crowns and roots where *Phytophthora* diseases causing root and stem rots normally originate (Rizzo et al., 2002). Furthermore, several species including *P. ramorum* and *P. infestans* are sometimes referred to as aerial blights since they attack twigs, stems, and other above ground structures (Werres et al., 2001). Aerial blights can be a significant problem in the production of horticultural crops (Denman et al., 2005). Diseases caused by *Phytophthora* have been associated with a major decline of Eucalypt forests in Australia (Podger, et al., 1965)

Taxonomy

Species of *Phytophthora* were traditionally classified as fungi in the Kingdom Fungi (Myceteae; former classification). Recent advances in phylogenetic research using modern molecular techniques has led to multiple and often conflicting classification revisions. *Phytophthora* has been classified as being a member of the Kingdom Chromista (Cavalier-Smith, 1986; Werres et al., 2001) with other classification systems further delineating a kingdom called the Stramenopila with the term Pseudomycota also being used (Patterson, 1989; Sogan and Hinkle, 1997). Stramenopila is gaining acceptance as a synonymous status with Chromista. However, pending further research, this review follows Cavalier-Smith's (Cavalier-Smith, 1986) classification using the hierarchy Chromista. The genus *Phytophthora* was first described by Anton deBary in 1876. The differences between the true fungi and the Chromista are based on evolutionary divergence, but plant pathologist have always considered *Phytophthora* to be 'fungus-like' and realized it had unique characteristics that needed to be considered when studying and controlling species within this genus (Erwin and Ribeiro, 1996). Indeed, it is thought that true fungi and members of the Chromista represent an example of convergent evolution (Cavalier-Smith 1987). The kingdom Chromista is characterized by having heterokont (different types of flagella) flagellation on zoospores and was formally named in 1986 (Cavalier-Smith, 1986). Chromista includes all protista having tripartite, hollow, tubular hairs (mastigonemes) arranged in two rows along the flagellar shaft and or a complete rough endoplasmic reticulum (RER) envelope around the chloroplast (Erwin and Ribeiro, 1996). The brown algae are included in the Chromista and are all thought to have evolved from a common ancestor (Erwin and Ribeiro, 1996). The Oomycetes (phylum to which *Phytophthora* belongs) evolved with and are phylogenetically related to heterokont algae (Erwin and Ribeiro, 1996). This concept is supported through ultrastructural similarities. Small subunit ribosomal RNA gene sequences agree that oomycetes differ at the molecular level from true fungi (Erwin and Ribeiro, 1996). The oomycetes are characterized by the production of zoospores with two cilia with anterior rootlets having a ribbed triplet and doublet with posterior rootlets having an octet and doublet and with cytoplasmic and nuclear associated microtubles.

The practical implication associated with whether *Phytophthora* is a true fungi or not is in the method of chemical disease control.

The following general taxonomic hierarchy of the genus *Phytophthora* is based on descriptions by Cavalier-Smith (1986), Bar (1992), and Dick (1969) with the understanding that this system may be supplanted by other systems upon consensus in the literature:

Kingdom: Chromista

Phylum: Oomycota

Class: Oomycetes

Order: Pernosporales

Family: Pythiaceae

Genus: *Phytophthora*

Characteristics of the Chromista are uni- or multi-cellular, filamentous, or colony forming microorganisms. They are primarily phototropic and some have tubular flagellar appendages and or chloroplast inside the rough endoplasmic reticulum. Fungi produce mycelium which contain glucans and chitin or chitosan, but lack chloroplasts (Cavalier-Smith, 1986, Tyler, 2001). Genomic analysis of RNA sequences show that fungi and oomycetes (Chromista) are phylogenetically distinct (Tyler, 2001).

Species of *Phytophthora* belong to the phylum Oomycota and are characterized by biflagellate zoopores. Longer tinsel flagellum are directed forward while shorter "whiplash" flagellum are directed backward. Cell walls are made up of glucans, hydroproline, and cellulose.

Oomycetes are fungal-like organisms that have elongated mycelium containing cellulose and glucans but have no cross walls. Oomycetes synthesize lysine via diamino-pimelate while fungi synthesize lysine via an alpha-amino-adipate pathway (Tyler, 2001). They produce in oogonium (female gametangium) zygotes called oospores. These are thick-walled spores and may be long-lived. Oomycetes also produce zoospores or zoosporania as their asexual spores. The Oomycota also produce chlamydospores (Dick, 1990; Barr and Desaulniers, 1990; Barr 1992).

Life History

Phytophthora species have coenocytic mycelium [coenocytic = pertaining to multinucleate mycelium in which the nuclei are not separated by cell walls] with no, or few, septa and in water produce zoosporangia that bear biflagellate zoospores [zoospores= spores that form within the sporangia and exits through a terminal pore, has a tinsel and a whiplash flagellum and are capable of swimming for several hours]. Sporangia are the sacs within which zoospores form, especially when water is cooled about 10° C below ambient temperature. In solid substrate, sporangia usually germinate by germ tubes. Zoospores can swim for hours but eventually cease swimming (Bimpong and Clerk, 1970). At this time, the spores round up or become spherical and within minutes develop a cell wall and is called a cyst (Bartnicki-Garcia and Wang, 1983). Encystment is induced by agitation, shaking, or spores naturally colliding with each other. Zoospores are thought to be a major source of infectious propagules (Erwin and Ribeiro, 1996).

Sexual oospores form singly within the oogonium after fertilization by a nucleus from the anteridium (Erwin and Ribeiro, 1996). The most important morphological feature of the oomycetes is sexual reproduction by production of oospores after union of two gametangia in which meiosis occurs prior to fertilization (Erwin and Ribeiro, 1996). The thallus is diploid (Erwin and Ribeiro, 1996).

Phytophthora, as a member of the Oomycetes, differs from other true fungi in a number of characteristics (Zentmyer, 1983; Griffith et al., 1992). The cell walls are composed of cellulose and beta-glucans but not chitin which is present in non-oomycetic fungi (Bartnicki-Garcia, 1969; Bartnicki-Garcia and Wang, 1983). The main storage carbohydrate is mycolaminarin, a beta-1-3 glucan (Wang and Bartnicki-Garcia, 1974). The zoospores are biflagellate with one being a whiplash and the other being a tinsel flagellum (Desjardins et al., 1969; Hemmes, 1983).

An important characteristic is that *Phytophthora* species do not synthesize sterols, but require an exogenous source of beta-hydroxy sterols for sporulation (Elliot, 1983; Hendrix, 1970). The Phythiaceae are resistant to polyene antibiotics such as pimaricin. This is coupled with an exogenous beta-hydroxy sterol requirement (Eckert and Tsao, 1962). Other fungi which synthesize sterols are sensitive to polyene antibodies. This

dependence on exogenous sterols offers strategies for the control of diseases caused by *Phytophthora.*

Inoculum from *Phytophthora* can range from undetectable to high levels in a very short time period (MacKenzie et al., 1983; Weste, 1983). The increase of inoculum is caused by the rapid production of sporangia and zoospores from infected plant tissue when environmental conditions are favorable. In particular, the presence of free water greatly increases spore production. Because of a short regeneration time and great reproductive capacity, diseases caused by *Phytophthora* are usually multicyclic or have the ability to reproduce many times during a single growing season (Fry, 1982; MacKenzie et al., 1983). The oospores produced are capable of surviving long periods of time and also show great genetic variability in the progeny. Multicyclic diseases can result in epidemics which quickly escalate when environmental conditions are favorable.

Production of sporangia and zoospores are the principal means by which the numbers of propagules are increased and account for rapid regeneration times. Time required for sporulation is species dependant and ranges from a few hours to a week (Erwin and Ribeiro, 1996) Sporulation physiology is complex and the induction in many soil borne species has exacting environmental combinations of moisture and temperature (Erwin and Ribeiro, 1996).

In laboratory studies using *Phytophthora in vitro* cultures, water free of metallic ions is essential. *Phytophthora* is sensitive to copper and possibly other metals (Kennedy and Erwin, 1961). Glass distilled water should be used to avoid metallic contamination (Gerrettson-Cornell, 1976). Copper concentrations as low as 1×10^{-7} M inhibited several species from sporulating (Halsall, 1977).

Soil borne species produce sporangia optimally on new mycelial growth only after the cultural media is changed from a nutrient rich to a nutrient poor media followed by water or salt solution rinses (Erwin and Ribeiro, 1996; Ho, 1969). Depletion of nutrients is an important factor in inducing sporulation of many species (Elliott, 1989; Yoshikawa and Masoago, 1977). Species that cause aerial blight sporulate under high relative humidity, while most root infecting species sporulate only in free water or other aqueous environments (Erwin and Ribeiro, 1996).

In addition to water, media pH can be important with most species favoring a neutral to acidic (pH 6 to 7) environment. Appropriate nutrient components must be present as well as an exogenous sterol source (Nes et al., 1982). Oxygen is also required and may be limiting in saturated soils (Erwin and Ribeiro, 1996). In addition, sporangium formation may be suppressed when carbon dioxide levels rise above ambient conditions (Mitchell et al., 1978). Light quality can also affect sporangium production on certain species. Light in the near-ultraviolet (320-400 nm) or blue (430-500 nm) region is favorable for sporangium formation in certain species (Erwin and Ribeiro, 1996). Many species, especially those which are soil-borne, may be inhibited by light or sporulate better in dark conditions (Gooding and Lucas, 1959; Harnish, 1965; Ribeiro et al., 1976).

As alluded to, many individual factors contribute to the sporulation process. Moreover, it is a precise combination of these factors that determine a specie's ability to successfully sporulate thereby producing a large number of propagules.

Zoospore production is the most important part of the *Phytophthora* life history as this is the mechanism that allows for rapid population increase and dispersal when free water is present. Many species (for example *P. infestans*) produce zoospores at lower temperatures (12º C) with direct germination of sporangium by germ tubes at higher temperatures (24º C). Many other species produce zoospores at higher temperatures as those species grow better at higher temperatures (Erwin and Ribeiro, 1996). In general, cooling sporangia cultures to 10º C below optimum growth temperatures stimulates zoospore formation (Erwin and Ribeiro, 1996).

Sporangium can germinate by production of germ tubes or by the production and release of zoospores. Sporangia that germinate by zoospore production are controlled by environmental factors such as temperature lowering and the availability of free water (Barr, 1992; Barr and Desaulniers, 1990; Hemmes and Hohl, 1971). When environmental conditions favor zoospore production, the cytoplasm in the sporangium cleaves around each nucleus and biflagellate zoospores form (Erwin and Ribeiro, 1996) Zoospores are expelled upon maturity from the sporangium by a difference in osmotic potential whereby water moves into the sporangium and increases turgor pressure within the sporangium (Gisi, 1983). A cap or plug consisting of a gel-like material dissolves

and the spores are pushed out of the apical opening by the higher internal osmotic pressure (Gisi et al., 1979; MacDonald and Duniway, 1978). Once released, the zoospores swim by means of their flagellates. After time, zoospores cease to swim and form a cell wall during the encystment process.

Phytophthora follows a typical disease life history. There are normally seven stages involved in a disease cycle and include the following (Agrios, 1997):

1. Inoculation
2. Penetration/germination
3. Establishment of infection
4. Colonization (invasion)
5. Growth and reproduction
6. Dissemination
7. Pathogen survival or over-seasoning

Inoculation occurs when the pathogen first comes into contact and enters the host plant. Inoculum consists of any part of a pathogen that can initiate infection once it is brought into contact with a host plant. A single unit of inoculum is a propagule.

Pathogens in a vegetative state can immediately infect a plant. Spores must germinate before they can penetrate plant tissue. Spore germination requires favorable temperatures and free water or at least high humidity. Ideal environmental conditions must persist until host plant penetration. Spores are normally capable of germinating immediately after maturation and release. Resting spores may require a dormancy period before germination is possible (Agrios, 1997).

Upon germination, spores produce a germ tube which can penetrate the host plant. Nutrients or other plant metabolites diffusing out of the plant's surface may stimulate or favor germinating spores (Agrios, 1997). Some pathogens germinate only by exposure to exudates of plants susceptible to that particular pathogen (Agrios, 1997). In other instances, spore germination may be inhibited by exposure to plant exudates (Agrios, 1997).

After germination, pathogens must penetrate the plant surface. This is accomplished by direct penetration through the surface or by penetrating natural openings (stoma, lenticels, hydathodes) in addition to plant surface wounds (Agrios, 1997).

Penetration does not always lead to infection. Pathogens which do penetrate directly do so by hypha produced directly by the spore or, though a penetration peg produced by an appressorium, which is a swollen tip of a hypha or germ tube that allows attachment facilitating penetration (Agrios, 1997). The hypha pierces the cuticle and cell wall through mechanical force and enzymatic action on cell wall substances (Agrios, 1997).

Infection is the process by which pathogens establish contact with susceptible host cells or tissue and procure nutrients (Agrios, 1997). Pathogens grow and or multiply within the plant tissue and spread or colonize surrounding tissue. Tissue invasion and pathogen reproduction (colonization) in or on infected tissues are two concurrent substages of disease development within the infection process. Infection results in the appearance of a particular pathogen's disease symptomology. However, some infections remain latent without symptoms until such time as environmental or other factors become favorable for disease expression. Fungal infection may be local and only involve a single cell or a few cells immediately surrounding the invasion site, or the infection may enlarge to cover plant organs (flower, leaf, fruit) or the entire plant (Agrios, 1997).

Fungi generally invade and infect tissue by growing into them from one point of inoculation (Agrios, 1997). These pathogens normally continue to grow and branch out indefinitely and spread throughout the affected tissue until infection stops or the plant is dead. Fungi causing vascular wilts invade plants by producing spores within the vascular system and the released spores are carried to other parts of the plant via sap or water flow (Agrios, 1997). This allows for subsequent infection away from the initial infection site. Most pathogenic fungi produce mycelia only within the plant they infect (Agrios, 1997). Few fungi produce mycelia on the plant surface (with the exception of the powdery mildew fungi: order Erysiphales Gwynne-Vaughan). The majority of fungi produce spores on or just below the surface of infected plants (Agrios, 1996). These spores are usually released outwards into the surrounding environment. Some fungi do not release their spores until the host plant dies and disintegrates (Agrios, 1996).

Pathogen propagules are disseminated by many methods. Wind, rain, insect, or animal/mechanical dispersions are common. The role of water in *Phytophthora* dispersal is especially important. Excess irrigation and rainfall are the most important factors affecting the severity and spread of diseases caused by *Phytophthora* (Agrios, 1997;

Erwin and Ribeiro, 1996). The duration of free water either in the soil or on the plant is important because of the favorable environment coupled with the high capacity of *Phytophthora* to reproduce by sporangia and zoospores (Erwin and Ribeiro, 1996). A main method of dispersal for zoospores or cyst (encysted zoospore) in soil is flowing water or rainfall splashing and runoff (Erwin and Ribeiro, 1996).

An important part in the disease life history is the ability of a pathogen to survive during periods unfavorable for growth and reproduction. Unfavorable conditions range from a lack of a suitable host to environmental conditions such as cold, heat, and extended drought. Several mechanisms have evolved to enable a pathogen to survive. A common survival mechanism is for mycelia or spores to remain on infected host plant tissues over time (Agrios, 1997). Such tissues include bud scales, decaying fruit, stem cankers, or infected leaves, stems, and roots. Annual or deciduous tissues that fall to the ground can be overseasoning refuges for pathogens (Agrios, 1997). Other pathogens may survive for long periods of time in the soil (Agrios, 1997). Additional survival strategies include overseasoning on seed, vegetative propagules, or insects (Agrios, 1997).

Phytophthora in the absence of host plants does not persist in the soil as long as other fungi that are saprophytic or possess the ability to colonize non-living organic matter (Agrios, 1997). Survival times for *Phytophthora* propagules vary greatly depending on individual species. Mycelia can persist from a few days to 2 years while chlamydospores or oospores may persist for six years under certain conditions (Agrios, 1997).

Phytophthora capsici: An Overview

Phytophthora capsici Leonian (1922)

syn. *P. hydrophila* Curzi

P. parasitica var. *capsici* (Leonian) Sarejanni

P. palmivora MF4 Griffin

Phytophthora capsici was first described by Leonian in 1922 (Leonian, 1922) as the causal agent of chili pepper (*Capsicum annuum* L.) blight in New Mexico. *Phytophthora capsici* is known to infect many plant species from temperate to tropical agronomic and horticultural crops worldwide. Diseases caused by *P. capsici* include foliar blights, fruit rots, stem and root rots (Erwin and Ribeiro, 1996).

Phytophthora capsici was re-described to accommodate a broad range of biotypes that were originally included under *P. palmivora* MF4. These isolates cause black pod of cocoa (*Theobroma cacao* L.) and black pepper (*Piper nigrum* L.) wilt (Tsao and Alizadeh, 1988; Tsao, 1991). Because *P. capsici* has been broadened in scope, host-specific pathogenicity is diverse. For example, Macadamia nut (*Macadamia integrifolia* L.) isolates are not pathogenic on pepper (*Capsicum* spp.) (Uchida and Araguki, 1989).

A world collection of 84 isolates were analyzed for isozyme relatedness. *Phytophthora capsici* is a genetically complex species containing three subgroups (Oudemans and Coffey 1991). Subgroup one, CAP1, contains isolates from annual solanaceous (Solanaceae Juss.) and cucurbit (Cucurbitaceae Juss.) species, as well as isolates from black pepper and cocoa previously described as *P. palmivora* MF4 (Kaosiri and Zentmyer 1980). Subgroup two, CAP2, contains isolates from mainly tropical crops such as black pepper, papaya (*Carica papaya* L.), macadamia, and rubber *Hevea braziliensis* (Willd. ex A. Juss.) Mull. Subgroup three, CAP3, mainly includes isolates from cocoa in Brazil and is the least genetically diverse (Oudemans and Coffey, 1991). Mchau and Coffey (1994) looked at 113 isolates of *P. capsici* and conducted further isozyme analysis. This analysis showed that the isolates could be separated into two subgroups, CAPA and CAPB. Each subgroup is diverse with a wide range of host and geographical distribution. Morphology varied in some members of the subgroups.

Phytophthora capsici needs a minimum of 10º C for growth with an optimal temperature of 28 ºC and a maximum temperature greater than 35º C (Stamps 1985). Studies report various temperature ranges under which growth will occur (Tsao, 1991; Leu and Kao, 1981). Isolates from CAP3 tend to grow better at higher (35º C) temperatures.

Sporangia are mostly papillate (nipple-shaped) with some showing semi-papillate forms. Sporangial shapes are influenced by light and cultural conditions (Tsao and Alizadeh, 1988; Tsao, 1991). The overall shapes range from subsperical, ovoid, obovoid, ellipsoid, fusiform (spindle-shaped), to pyriform (pear-shaped). Sporangia are tapered at the base and are cauduous (from the Latin "to fall") on a long pedicel (Erwin and Ribeiro, 1995). Caudicity, or the ability of sporangia to readily detach from sporangiophores and be carried by wind currents is a primary method of dispersal (Erwin and Ribeiro, 1995). Mchau and Coffy (1994) also report much variability in morphology. Sporangiophores formed under light are irregularly branched and sympodia (chains) form only in water. Isolates of subgroup CAPA produce rounded ellipsoid sporangia, some with multiple papilla. Subgroup CAPB produce ellipsoid-lanceolate sporangia. Morphology can be diverse among isolates (Erwin and Ribeiro, 1995).

According to Tucker (1931) *P. capsici* from annual pepper rarely produce chlamydospores in culture. Ristaino (1990) reports no chlamydospore formation from annual pepper or cucurbit isolates. Some chlamydospores were reported on annual peppers grown in Iran (Ershad, 1972). Mchau and Coffy (1994) suggest chlamydospore formation is related to isolates from subgroup CAPB. Chlamydospores were observed to be terminal or intercalary. Chlamydospore production in some isolates is determined to some extent by cultural methods (Uchida and Araguki, 1985). Mycelial mats grown for five days on clarified vegetable extract juice (V8) and then submerged in sterile distilled water and incubated in the dark produced chlamydospores in 20 of 29 *P. capsici* isolates. However, with the exception of three isolates from eggplant (*Solanum melongena* L.), isolates from species of Solanaceae did not produce chlamydospores (Erwin and Ribeiro, 1995).

CHAPTER IV
PHYTOPHTHORA CONTROL: THEORY AND STRATEGY

Overview

Effective *Phytophthora* deBary control strategies must take into account factors that contribute to disease development. Successful strategies should achieve as much disease control as is efficient and cost effective. This is most effective in control of *Phytophthora* by focusing on the weakest link in the pathogen's life history.

To begin, a brief discussion of disease causing factors is necessary. These factors include an organism capable of causing disease (pathogen), a susceptible host, and a suitable environment in which the pathogen can successfully complete its life history or otherwise inflict harm on its host by disease manifestation. The degree of interaction between these factors determines the severity of a disease (Agrios, 1997). A classic example representing the interactivity of these factors is the concept of a disease triangle (Agrios, 1997). Each side of a theoretical equilateral triangle represents one of the above mentioned factors. Creating conditions or situations where one of the three sides of the triangle is adversely affected reduces the incidence or the severity of the disease. A more accurate method of describing this interaction is thinking of each side of the three factors as a separate circle or sphere. Bringing the three circles into proximity from partial to complete overlapping represents a degree of disease severity (Erwin and Ribeiro, 1996). Complete separation of the circles indicates a condition where no disease occurs. A partial overlapping indicates a condition where an endemic or low threshold of disease occurs. A complete overlapping of the three factors indicates a condition where epidemic disease is possible. Any strategy which can theoretically move the circles further apart will reduce the incidence of disease (Erwin and Ribeiro, 1996).

When considering the theoretical disease triangle and the three factors involved (pathogen, host, and environment), environment, which in this instance includes plant culture, is one factor that can be modified, particularly in container or greenhouse production. Environment by this definition can include not only climatic factors such as rainfall, temperature, or humidity, but other diverse elements including irrigation or water

management, soil or other growing substrates, abiotic and biotic stresses, and host plant nutritional status. These elements can influence the severity and spread of diseases. Specific factors reported to increase the severity or spread of diseases caused by *Phytophthora* are listed as follows:

1. Water saturated soil (Bowers et al., 1990; Bowers and Mitchell, 1990)
2. Soil hardpans (Shea et al., 1984; Zentmyer, 1980)
3. Drought stress (Blaker and MacDonald, 1986; Ristaino and Duniway, 1989)
4. High soil salinity (Blaker and MacDonald, 1986; MacDonald, 1982, 1984)
5. Increase in soil pH (Kincaid et al., 1970)
6. Leaf wetness (Grove et al., 1985; Kuske and Benson, 1983)
7. Mound building ants (McGregor and Moxon, 1985)
8. Excess fertilization (Elliott, 1989; Hoitnick et al., 1986; Utkhede, 1984)

The two most important factors in the severity and spread of diseases caused by *Phytophthora* are excessive irrigation and rainfall (Erwin and Ribeiro, 1996). Rotem and Palti (1969) reported that flood irrigation and sprinkling foliage has an important impact on the incidence of disease.

Free water, either in the soil or on the plant foliage or fruit, for a prolonged time is the most important environmental consideration as this allows for a favorable situation for the rapid increase in the production of *Phytophthora* inoculum (Grove et al., 1985). Free water allows the reproductive capacity of *Phytophthora* to rapidly increase the formation of sporangia and zoospores. Zoospores and cysts are normally moved in the soil through flowing irrigation water, rainfall runoff, and physical soil movement by any method. Reports from Australia show that soil infested with *Phytophthora cinnamomi* Rands used to construct new roads in Jarrah (*Eucalyptus marginata* J. Donn *ex* Sm.) forests contributed to a pattern of diseases caused by *Phytophthora* that subsequently developed along those forest roads (Shea et al., 1983; Weste, 1983).

Zoospores are motile, but independently cannot move very far in soil. However, in flowing water, zoospore movement is widespread (Erwin and Ribeiro, 1996). References cite instances of *Phytophthora* being isolated in bodies of water such as canals, reservoirs, lakes, and streams (Garber et al., 1986; Klotz et al., 1959; von Broembsen, 1984). Excess irrigation in containerized plant production is also common

(Erwin and Ribeiro, 1996). Although containers normally have drainage holes, drainage is much less efficient than in field situations. Substrates may actually stay saturated for a prolonged time particularly at the bottom of the containers. Additionally, containerized plant production often involves the use of overhead sprinkler irrigation which in turn leads to prolonged wet foliage and high humidity immediately surrounding the container.

Free water in the soil will increase the number of sporangia and or zoospores which can increase the occurrence and severity of disease (Mitchell et al., 1978). In areas of low or seasonal rainfall, irrigation management can be a first line of defense in both field production and containerized nursery operations. This also applies to greenhouse crop production where rainfall is not usually a factor and irrigation is controlled. A paramount factor that is decisive in the severity of root disease is the length of time that soil remains saturated or near saturation (Duniway, 1983; Pfender et al., 1977). A confounding factor is that flooded soils can set up an anaerobic (anoxia) condition that causes root rot conditions which often mimic diseases caused by *Phytophthora* (Erwin and Ribeiro, 1996).

As mentioned, the life history of *Phytophthora* requires free water for the spread of zoospores. Zoospores have the ability to stick to plant surfaces because of the rapid production of an adhesive substance during the encystment process (Sing and Bartnick-Garciai, 1975). This adhesive substance is thought to be associated with lectin-ligand interactions and is not host specific (Hardham et al., 1991; Hohl, 1991). In orchards or other crop areas with permanent established plantings, such as landscapes, inoculum of *Phytophthora* cannot be economically eradicated. Cultural practices should be such that growing conditions are plant favorable, but not favorable for the pathogen. One of the most important methods for the prevention of crown rot on tree trunks is to prevent irrigation water from splashing or spraying on the trunks, or limiting overhead irrigation on nursery sites (Klotz, 1978).

A soil hardpan, or any change in soil texture, below the surface can create an interface which affects the percolation of water through the soil profile (Erwin and Ribeiro, 1996). This interface may be temporary as in the case of sandy soils, or permanent in the case of heavy clay or a concreted lateritic layer (hardpan). While this is a common condition in field soils, especially where heavy equipment is in continual use,

a similar boundary layer effect can occur in containerized plants with bottom drainage holes (Erwin and Ribeiro, 1996). A permeable growing substrate at the container interface will cause a temporary impedance to the downward flow of water. If this condition extends the length of time that the substrate remains saturated, this can set up an environment favorable to sporangia development and zoospore release.

To illustrate the affect of a clay hardpan in a native soil contributing to the development of a specific epidemic caused by *Phytophthora*, it is useful to look at a case described by Shea and others (1983) previously mentioned concerning the decline of a Jarrah forest in Western Australia from 1965 to the mid-1980's. The conundrum was to explain how large Jarrah trees, whose death was attributed to *Phytophthora cinnamomi*, died during the dry season when the trees had large vertical roots extending deep into the ground which should have supported the trees during the dry season. All data indicated that the environment was only marginally favorable for the development of *Phytophthora cinnamomi*. Although fibrous roots were lost to *Phytophthora cinnamomi* at very shallow layers (< 10 cm) during the wet season, it was difficult to explain such widespread damage during the dry season when soil sampling showed a low population of *Phytophthora cinnamomi* and large vertical roots appeared to be intact. Research showed that even in years with above average rainfall, *Phytophthora cinnamomi* could not be consistently detected in the fibrous root zone near the surface. Shea concluded that sampling methodology was not looking in the right location. When subsequent dead trees were excavated to a depth below the concreted lateritic layer (hardpan), lesions typical of *Phytophthora cinnamomi* were immediately recognized along the large vertical roots that penetrated the hard pan some 10 to 70 cm below the soil surface. Repeated root system excavations were able to isolate *Phytophthora cinnamomi* from where large roots extended into the hardpan and left cavities. Ultimately, it was shown that Jarrah trees produce strong vertical roots which penetrate the hardpan layer leaving channels and holes. Water percolating down through the soil profile collected in these channels and holes and was prevented from further downward movement due to the impervious soil layer. This set up a water saturated zone where *Phytophthora cinnamomi* proliferated in sporangium and zoospore production. This in turn caused infection of the large roots to take place producing the characteristic lesions on the vertical roots. These

damaged roots were unable to transport water upward from lower reservoirs of water during the dry season causing subsequent die-back and eventual death. Shea et al. (1983, 1984) found *Phytophthora cinnamomi* where roots penetrated the hardpan at depths (> 70 cm) not previously reported. Additionally, they found high sporangium development at the hardpan interface only because of the high retention of soil water (Shea, et al., 1983). Subsequent research in Jarrah production and forest management shifted focus from fine root susceptibility to studies focusing on root and root collar infections (Shea et al., 1982).

Environmental factors of a non-genetic nature that can affect a plant's disease susceptibility prior to infection is known as predisposition (Schoenweiss, 1975). Predisposition is an important concept because many plant genotypes thought to be disease resistant may become susceptible under adverse or predisposing conditions. A predisposing effect is different than a simple environmental factor, such as a high rainfall event, which may cause an increase in pathogen inoculum, but does not necessarily affect the host plant. Main factors predisposing a plant host to disease include, drought stress, pre-inoculation flooding, and soil or water salinity (Duniway, 1983).

Field crops such as safflower (*Cathamus tinctorius* L.) and tomato (*Solanum lycopersicum* L.) show an increase in *Phytophthora* root rots following drought stress (Knowles et al., 1965; Ristaino and Dunway, 1989; Zimmer and Urie, 1967). Even disease resistant cultivars of safflower became susceptible when exposed to drought stress (Duniway, 1977). The exact mechanism that triggers this effect is complex and not fully known, but may relate to net photosynthesis or possible desiccation of roots and corresponding release of amino acids into the soil (Hsiao, 1973; Katznelson et al., 1955; Ristaino and Dunway, 1989; Sharp and Davies, 1979).

Ornamental species produced in containers may also show a predisposition to disease susceptibility from water or salinity stress. Rhododendron (*Rhododendron* L.) produced for retail sale are predisposed to root rots by *P. cinnamomi* by water stress and salinity (Blaker and MacDonald, 1981). The susceptible cultivar *Rhododendron* 'Purple Splendor' did not show an increase of disease incidence when placed under drought stress and then inoculated; however, the resistant cultivar *Rhododendron* 'Caroline' showed a

marked increase in disease incidence after exposure to pre-inoculation water stress (Blaker and MacDonald, 1981).

Chrysanthemum (*Chrysanthemum* x *morifolium* T. de Ramatuelle) placed under artificial salinity stress (NaCl at 0.1 and 0.2 M soln.) were later inoculated with zoospores of *Phytophthora cryptogea* Pethybridge and Lafferty. Salt stressed plants had 70-88% of their roots showing lesions with non-stressed plants showing a 20% lesion cover (MacDonald, 1982, 1984). Swiecki and MacDonald (1988) looked at tissue penetration of *P. cryptogea* on chrysanthemum roots pulsed with NaCl in a nutrient solution for 24 h and then inoculated with zoospores. Results showed that salt stressed roots were deeply penetrated while non-stressed roots were only penetrated to a depth of 3 to 4 cells within 6 to 12 h after inoculation. Photographic microscopy of the infection process indicated that salinity inhibited root defense systems (Swiecki and MacDonald, 1988). While phytoalexins (plant produced antimicrobial substances) have not been shown to play an active part in chrysanthemum resistance, salinity repressed the production of gyceollin; a phytoalexin in soybean [*Glycine Max* (L.) Merrill] (Murch and Paxton, 1980). While high soil salinity increased the severity of root rot of tomato caused by *P. cryptogea*, asexual reproduction of *Phytophthora nicotianae* Breda de Haan (syn. *P. parasitica* Dastur) was suppressed (Swiecki and MacDonald, 1991). Snapp and others (1991) concurred that high soil salinity increased the severity of tomato root rot by *P. nicotianae*. High soil salinity increased the severity of stem rot caused by *Phytophthora citrophthora* R. E. Smith and E. H. Smith on numerous citrus (*Citrus* L.) rootstocks. High salinity also reduced the production of the phytoalexin 6, 7-dimethoxycoumarin in citrus roots (Sulistyowati and Keane, 1992). Weicht and MacDonald (1992) concluded that the possibility of root infection should be taken into account when breeding plants for salinity resistance.

In contrast to drought predisposing plants to disease, flooding soils prior to inoculation increased root rot in alfalfa (*Medicago sativa* L.) from *Phytophthora medicaginis* E.M. Hansan and Maxwell (Kuan and Erwin, 1980). Flooded or otherwise water saturated soils reduces the amount of oxygen available to roots. Soils flooded for three days showed alfalfa roots to be much more attractive (chemotaxis) to *Phytophthora medicaginis* zoospores than in non-flooded soils (Kuan and Erwin, 1980). Sampling of

root exudates indicated increased electrical conductivity and higher concentrations of sugars and amino acids indicating that root leakage increased on flooded soils. Aerenchyma (tissue with large air spaces) production is common in plants exposed to prolonged flooding (Drew and Lynch, 1980). Flooded conditions causes an elevation in soil ethylene production which in turn increases the formation of arenchyma tissue in the xylem (Drew and Lynch, 1980). Ethylene caused alfalfa roots to become more susceptible to *P. medicaginis* (Zook and Erwin, 1986).

A depleted soil oxygen level associated with an increased incidence of root rots caused by *Phytophthora* has been found in loblolly pine (*Pinus taeda* L.) and shortleaf pine (*Pinus echinata* Mill.) (Fraedrich and Tainter, 1989). Cherry (*Prunus* L.) also showed predisposition to root rot by *Phytophthora* from flooding (Wilcox and Mircetich 1985). Conversely, seedlings of Fraser fir [*Abies fraseri* (Pursh) Poir] were not predisposed by pre-inoculation flooding (Kenerley et al., 1984). However, this lack of predisposition in Fraser fir may indicate that either the host was greatly susceptible to *Phytophthora cinnamomi* in the first place, or that isolate was particularly virulent or else the population was so high as to overshadow the predisposing factor. Additionally, stems of *Eucalyptus sieberi* L.A.S. Johnson were more susceptible to *Phytophthora cinnamomi* when under a high plant water potential than when under drought conditions (Smith and Marks, 1986).

In addition to drought and flooding predisposing plants to disease, other factors can play a role. MacDonald (1991) showed that container grown chrysanthemum exposed to a growing substrate temperature of 45 to 47° C increased disease incidence from *Phytophthora cryptogea* in roots. A 30 min. exposure to substrate temperature of 40 to 45° C showed a marked increase in disease incidence. The response was similar in containers and hydroponic culture (MacDonald, 1991).

Transplants of Strawberry (*Fragaria* x a*nanassa* Duchesne) injured by freezing temperatures were more susceptible to crown rot caused by *Phytophthora cactorum* (Leb. and Cohn) Schroeter than from uninjured transplants. Uninjured plants only became susceptible upon artificially wounding the plants (Erwin and Ribeiro, 1996).

Biotic factors such as nematode [*Meloidogyne incognita* (Kofoid and White) Chitwood] infection can increase the incidence and severity of root rots caused by

Phytophthora in alfalfa plants (Welty et al., 1980). A similar situation has been found in tobacco (*Nicotiana tabacum* L.) cultivars resistant to *P. nicotianae*. Resistance was overcome when roots were injected with *M. incognita*, the organism that causes root knot (Powell, 1979; Powell and Nusbaum, 1960). Finally, potato (*Solanum tuberosum* L.) leaf age can affect predisposition to infection by *Phytophthora infestans* (Mont.) deBary. As leaf age increased, the degree of infection decreased. However, the ability of *P. infestans* to sporulate increased slightly in older leaves and plants (Lapwood, 1961a; Lapwood, 1961b; Lowings and Acha, 1959; Rotem and Sari, 1983).

Research on environmental factors as related to disease incidence and severity has tended to focus on the pathogen and not as much on the host plant. The rationale is that whatever happens to reduce the pathogen population will reduce the incidence of disease. Research on predisposition suggest that this reasoning may be partly false and that factors that affect the condition of the host plant should be considered (Duniway, 1983; Fraedrich and Tainer, 1989). The assumption that root disease caused by *Phytophthora* can be controlled simply by allowing a substantial reduction in irrigation frequency and quantity may not be a safe or valid assumption. Maintaining a delicate balance between environmental conditions that are not extreme plays an important role in control of *Phytophthora*l.

When a specific host plant is absent, most species of *Phytophthora* do not survive as long as other types of fungi that are more saprophytic such as some species of *Pythium* Springsheim or *Fusarium* Link *ex* Gray. However, many species of *Phytophthora* can persist in soil for long periods of time meaning that the absence of a host plant does not indicate total eradication of that pathogen. Chlamydospores of *P. cinnamomi* were found to have survived up to six years (Hwang and Ko, 1978). Although data suggest that propagules of *Phytophthora* survival times are relatively short, accessing long term survival potentials may be obscured by the difficulty of successfully detecting various species of *Phytophthora* in the soil. The ability of a relatively small number of propagules to increase rapidly under favorable conditions may compensate for short survival times (Erwin and Ribeiro, 1996)

Effective control strategies are related to the ability of each species of *Phytophthora* to survive in the absence of a host plant or favorable environment. Much

variation exists among species of *Phytophthora* survival potential either as saprophytes or dormant spores. Generally, mycelium and zoospores survive for only a few weeks in the absence of a host. Chlamydospores, however, may survive much longer and some oospores may persist up to thirteen years (Erwin and Ribeiro, 1996). Such long-term survival precludes a pathogen such as *Phytophthora cinnamomi* from being controlled by crop rotation or allowing a field to lay fallow for a reasonable amount of time (Zentmyer and Mircetich, 1966). Additionally, Zentmyer (1980) and Reeves (1975) suggests that *Phytophthora cinnamomi* may be a competitive saprophyte. *Phytophthora medicaginis* is known to colonize organic material in the soil and produce oozpores even in soils at low temperature (Stack and Millar, 1985). *Phytophthora palmivora* Butler, conversely, is thought to be a poor saprophyte since it only migrates a short distance in the soil and then lyses (Ko, 1971; Sneh and McIntosh, 1974; Tsao, 1969). The mechanism by which differing species overwinter (or overseason) varies by the various types of propagules that are able to survive. Gregory (1983) studied several disease epidemics including late blight of potato (*Phytophthora infestans*), black pod on cacao (*Theobroma cacao* L.) caused by *Phytophthora megakarya* Brasier and Griffin, root rot caused by *Phytophthora palmivora* on *Eucalyptus* in Australia, leaf blight caused by *Phytophthora colocasiae* on Taro [*Colocasia esculenta* (L.) Schott], and finally fruit rot of apple (*Malus* L.) by *Phytophthora syringae*. Each case showed a different type of propagule initiated that particular epidemic. *Phytophthora infestans* survived by mycelium in infected tubers and also in the soil for short periods although the propagule for the soil inoculum was unknown. *Phytophthora colocasiae* produced both chlamydospores and oospores in taro rhizomes. In apple, *Phytophthora syringae* produced oospores in leaves that were later deposited on the ground. In cacao, *Phytophthora palmivora* produced chlamydospores in infected fruit. *Phytophthora megakarya* was thought to survive in infected cocao flower parts and possibly in infected but asymptomatic roots (Gregory, 1983). *Phytophthora cinnamomi* survived as chlamydospores in infected taro roots as well as in the ground (Weste, 1983).

Erwin and Ribeiro (1996) concluded that most likely all root, crown and foliar diseases caused by *Phytophthora* are multicyclic since these diseases can be initiated from low, or even undetectable, levels of inoculum and that pathogen levels can increase

exponentially if conditions are right. Propagule longevity depends on the ability to colonize plant or other organic material and to persist in gravel, sand, or soil independent of a host (Weste, 1983). Major factors that contribute to the survival of *Phytophthora* in soil are temperatures sub-optimal for plant growth, adequate soil moisture, and low soil microbial activity. An increase in fungi and bacteria populations can suppress propagules of *Phytophthora* (Weste, 1983). As previously mentioned, surviving propagules (oospores, chlamydospores, and mycelium) under favorable conditions can quickly germinate or otherwise grow and produce sporangia and zoospores thereby quickly increasing inoculum (Weste, 1983). The entire process from germination to zoospore production requires less than 48 to 96 h under optimum conditions. Therefore, "explosions" of inoculum from *Phytophthora* can rapidly cause subsequent epidemics (MacKenzie et al., 1983).

The transportation of infected plant parts or soil into previously uninfected areas is the main method of long range dispersal of *Phytophthora*. A historic example is the spread of late potato blight *Phytophthora infestans* worldwide through the shipping of infected potato tubers used to propagate plants. As pointed out earlier, trucks and other road building equipment in addition to fill soil, were shown to be a factor in the spread of root rot caused by *Phytophthora cinnamomi* in Jarrah trees in Western Australia (Shea, 1988). Currently of concern is the spread of *Phytophthora ramorum* Werres, DeCock, and Man in't Veld via shipment of nursery stock from infected areas to pathogen free areas (U.S.Depatment of Agriculture, 2005).

An important advantage in spore or inoculum dispersal in many species of *Phytophthora* is a morphological adaptation called caducity. Caducous species have the ability to detach sporangia from the sporangiophore much like the abscission zone that forms during leaf senescence. This ability to detach readily allows spores to become airborne moving on air currents and wind, and also moved by rainfall or splashing water. Gregory (1983) refers to such caducous sporangia and zoospores as xenospores (from the Greek *Xeno* meaning guest or stranger). The ability to be moved with ease is an evolutionary advantage for some species of *Phytophthora* over other microorganisms. In addition to abiotic factors such as rainfall runoff, splashing, and wind, biotic factors, other than humans, such as ants and rats can transport inoculum (Erwin and Ribeiro,

1996). Pruning shears and other production equipment and practices can also spread inoculum (Newhook and Jackson, 1977).

Up to this point, biotic and abiotic factors as they relate to the ability of *Phytophthora* to infect a susceptible host have been reviewed. Such factors include the interaction of the pathogen, host, and environment. Soil, water management, and soil conditions were mentioned. A plant's genetic disposition to disease was found to be an important factor in some plant species. And finally, inoculum survival and dispersal were reviewed. The next section will explore strategies to control diseases caused by *Phytophthora*. The control strategies include such areas as cultural practices, biological control, host resistance, and chemical control. Effective control of *Phytophthora* is usually a combination of several strategies and practices.

The prevention of a disease ever getting a foothold is the best defense against plant damage and potential epidemics. A referral back to the concept of the disease triangle indicates that eliminating either the pathogen (source of inoculum), host plant, or a favorable environment will prevent, or substantially reduce, the incidence of disease. This principle holds true whether control is desired locally on-site, or world-wide. Total elimination or isolation of infectious organisms would be desirable, but rarely obtainable. Modern transportation systems and global economies make "walling off" vulnerable agricultural or other plant production systems very difficult (Erwin and Ribeiro, 1996). Notwithstanding the difficulties of preventing the spread of pathogens from infected to non-infected areas, quarantines still play an important role in governmental attempts to control the spread of specific pathogens. Currently, a major component of the US Department of Agriculture's national strategic plan (U.S. Department of Agriculture, 2005) for control and management of *Phytophthora ramorum* relies on quarantining infected areas and preventing the movement of susceptible plant species (in this case containerized nursery stock) into non-infected areas of the country. This program also relies on the eradication of infected material (host elimination) and remediation of affected forest areas (environment manipulation). *Phytophthora ramorum* is particularly problematic since it infects a wide number of disparate hosts. These hosts include not only ornamental plants produced in containers, but also many species occurring in forest, parks, and other natural habitats. The attempted control of *Phytophthora ramorum* is of

national and international concern and is an example of a widespread and multi-organizational effort (U.S. Department of Agriculture, 2005). The same principles of isolation, eradication, and remediation are useful tools to the individual producer to prevent the spread of diseases locally on-site or in the immediate area.

Sanitation is the process of preventing the deposition of inoculum and removing any plant material suspected of being infected. In field production situations, this process is effective for the control of epidemics from mono-cyclic diseases that produce only one cycle of inoculum during the growing season, usually in autumn (Vanderplank, 1963). Sanitation is less effective in the field when diseases are multi-cyclic such as many species of *Phytophthora* that cause foliar blights. Planting disease-free seed potatoes delayed, but did not prevent, epidemics later in the season (Bonde and Schultz, 1943, 1944; Vanderplank, 1963). Sanitation alone is insufficient to control *Phytophthora infestans* since the organism is capable of rapid regeneration from small residual populations of inoculum. MacKenzie and others (1983) suggest that multicyclic diseases are hard to control in field or orchards through sanitation alone. However, control of *Phytophthora cinnamomi* in Australia by washing trucks and equipment has been an effective control strategy (Shea et al., 1983).

Conversely, sanitation is very important in nursery and greenhouse production facilities in reducing disease incidence. Forsberg (1985) isolated numerous species of *Phytophthora* from ornamental plants in production. Forsberg recommends that ornamental plant nurseries adopt an accreditation system similar to the one used by the avocado (*Persea americana* Mill) industry (Pegg, 1978) to insure the production of disease free stock plants. While sanitation is very important in nursery and greenhouse production to reduce the amount of inoculum from infected plants or equipment, research indicates that irrigation water can be a source of inoculum of *Phytophthora* (MacDonald et al., 1994; Ribeiro and Linderman, 1991; Taylor, 1977) A vigorous program to insure pathogen-free stock material from propagation to shipping will greatly reduce the potential for disease outbreak.

Steam heat has traditionally been used to sterilize soil or other growing substrates on a limited scale. While very effective, it is also expensive and not practical on a large scale, or even desirable. The standard method is to use pressurized steam to heat the coolest part of the soil to at least 82º C for 30 min. with the idea that the soil will eventually reach 100º C (Erwin and Ribeiro, 1996). It must be kept in mind that the sterilization process, by definition, will eliminate all microorganisms including those antagonistic to species of *Phytophthora*; hence, the question of desirability for sterilized soil. Re-introduction of inoculum from disease causing organisms into a sterile substrate can very quickly lead to an explosion of pathogenic inoculum if conditions are favorable.

Another method that has been found useful in reducing the incidence of *Phytophthora* in field soil or landscape ground beds is the process of solarization. The principle is to cover the soil, which must be moist (Pullman et al., 1979), with a clear plastic tarp or film trapping the heat produced by the infrared radiation from the sun. This method is effective where summers are hot and cloudless. Solarization's effectiveness in controlling soil borne microorganisms was first described by Katan and others (1976). Several researchers have further described the methodology and effects of solarization (Katan et al., 1976; Katan and DeVay, 1991; Stapleton and DeVay, 1982, 1986).

Thermal inactivation is another method to using heat to control pathogens in infected or otherwise susceptible plant material. This is based on the fact that some plants, or plant parts, can survive thermal temperatures higher than propagules of *Phytophthora*. Hot water soaks of calla lily [*Zantedeschia aethioica* (L.) K. Spreng.] at 50° C for 1 h suppressed *Phytophthora richardiae* Buisman (Dimock and Backer, 1944). A 2 min. soak at 48.9º C eliminated *P. citrophthora* R.E. Smith and E.H. Smith from infected lemon [*Citrus lemon* (L.) Burman] fruits (Klotz, 1940; Klotz and DeWolfe, 1961). Dormant grape cuttings (*Vitis* L). have been treated with hot water soaks to reduce pathogen level prior to rooting (McEachern, 2006).

The rationale for crop rotation is that monoculture of a specific crop on the same site will eventually lead to a situation where pathogenic inoculum builds to a level where disease incidence becomes a limiting factor in production (Glynne, 1965). While this is normally the case, exceptions exist. Most orchard crops are monoculture systems that co-

exist for decades on soils infested with *Phytophthora*. Examples include apple, and citrus orchards. However, other fruit bearing trees, such as avocado can be susceptible on marginal sites where even large trees can succumb to *Phytophthora cinnamomi* over time (Zentmyer, 1980). Crop rotation is an important strategy for annual crops. Some species of *Phytophthora* are subject to microbial antagonism. While there is evidence of long term survival of oospores and chlamydospores in the soil, *Phytophthora* is not a strong competitive saprophyte (Tsao, 1969) and would not normally persist in a high population without a host plant. Crop rotation can also be successfully employed in landscape situations and ornamental plant production facilities. This may be especially important where seasonal "color beds" are often repeatedly planted to the same species yearly thereby allowing for a re-infection to occur.

Conventional thought suggests that a well fertilized vigorously growing plant is more disease resistant than a nutritionally starved plant. While this is true in many cases, evidence suggests that certain species of vigorously growing plants may actually be more susceptible to disease (Erwin and Ribeiro, 1996).

Nitrogen is usually the most commonly applied nutrient in growing agronomic crops since many times it is a limiting factor in plant growth. Schmitthenner and Canady (1983) suggest that nitrogen has both a positive and negative impact on diseases caused by *Phytophthora*. This research shows that the form of available nitrogen can increase or decrease the incidence of disease (Schmitthenner and Canady, 1983). Luxury amounts of nitrogen increase succulence which can increase susceptibility. *Rhododendron* supplied with high levels of nitrogenous fertilizers became more succulent and more susceptible to leaf blight caused by *P. cactorum* (Hoitink et al., 1986). The most effective use of nutrition management to reduce disease incidence is to reduce soil pH. Sulfur additions to acidify the soil to pH 3.8 drastically reduced pineapple [*Ananas comosus* (L.) Merrill] root rot caused by *P. cinnamomi* by acting to supress the formation of sporangia in soil (Pegg, 1977). While reduction to pH 4.0 by sulfur or aluminum sulfate fertilizers suppresses many species of *Phytophthora*, this is obviously useful only on plants adapted to low pH soils (Pegg, 1977; Schmitthenner and Canady, 1983).

The chemical element involved may be as important as the absolute pH value. Treatment of acidic (pH 4.8) greenhouse soils with calcium carbonate and calcium

hydroxide to raise soil pH increased the incidence of seedling blight caused by *Phytophthora capsici*. The increase in disease incidence was attributed to an immobilization of free aluminum instead of a simple rise in pH (Muchovej et al., 1980). Aluminum applied as a soil drench controlled seedling blight caused by *Phytopthora. parasitica* on periwinkle [*Catharanthus roseus* (L.) G. Don] grown in an inoculated peat-based substrate (Bensen, 1993). The addition of calcium carbonate to buffer soil pH increased the amount of aluminum sulfate needed to control seedling blight. However, an increase in soil pH actually reduced the amount of exchangeable elemental aluminum (Al^{+3}) needed to control blight. Bensen (1993) suggests that the fungistatic effectiveness of aluminum is caused by the presence of soluble aluminum. Additionally, it is suggested that the addition of calcium to aluminum sulfate could reduce the affect of aluminum toxicity while still allowing for fungistatic control of *Phytophthora parasitica*. High pre-plant levels of nitrogen applied as ammonia (NH_4^+) suppressed *Phytophthora cinnamomi* and *Phytopthora parasitica* (Tsao and Oster, 1981). The downside is that high levels of ammonia applied fertilizers can be phytotoxic (Gilpatrick, 1969).

The mechanisms by which nutrients suppress *Phytophthora* are not known. In fact, investigations into the role of various nitrogenous fertilizers show contradicting results (Schmitthenner and Canady, 1983). This research concluded that multiple factors such as soil type, host pathogen interactions, and nitrogen formulation determined disease severity.

Peat-based substrates are commonly used to grow containerized plants in greenhouse and nursery operations. These peat-based substrates are well drained and are of great value in the production of containerized plants (Baker, 1957). While peat moss is acidic (pH 4.0), its use in growing substrates does not lower pH enough to suppress most soil pathogens. An economic alternative to peat moss is the use of composted hardwood bark (Daft et al., 1979; Hoitink, 1980; Hoitnick and Fahy, 1986; Hoitink and Powell, 1990). Composted hardwood bark has been shown to suppress rhododendron root rot caused by *Phytophtora cinnamomi* in soil (Gerrettson-Cornell et al., 1976). Composted bark can be effective in controlling *Phytophthora* in containers as it reduces the need for soil sterilization or fungicidal drenches (Hoitink and Powell, 1990). Mechanisms of controlling *Phytophthora* by hardwood compost were reviewed by

Hoitink and Fahy (1986). This review suggests that hardwood bark decomposes more slowly than pine bark and that composting is an aerobic process that generates internal heat to 60 to 70° C. Pathogens are suppressed by high temperatures, toxic by-products from decomposition, and by re-colonization by microbes antagonistic to *Phytophthora* (Hoitink, 1986; Hoitink et al. 1976). The natural process of decomposition releases chemical inhibitors of sporangia and zoospores (Hoitink, 1986; Hoitink et al., 1976). This chemical inhibition usually only last about a year (Hoitink and Powell, 1990). Leachates from the composted material also have been shown to suppress *Phytophthora* (Spring et al., 1980).

There is a limited amount of data on the effects of organic amendments on suppression of *Phytophthora*. Linderman (1989) reports that certain soil amendments can either increase or suppress certain diseases. The mechanisms for the control and suppression are not fully known (Linderman, 1989). Amendments known to have an adverse affect on *Phytophthora* in greenhouse systems include alfalfa meal, cotton waste, soybean meal, wheat straw, chicken manure, hydrolized feather meal, and urea (Gilpatrick, 1969; Tsao, 1977; Tsao, and Zentmyer, 1979; Zentmyer, 1983). Use of amendments has not shown consistent results. In general, these compounds are thought to work via breakdown products which may suppress sporangia formation, reduced zoospore germination, or reduced mycelial growth (Zentmyer and Thompson, 1967).

Biological control of *Phytophthora* is not a single mode of action or procedure, but rather a complex combination of components that interact to create a desired effect. Indeed, trying to define biological control can be problematic. One definition is the reduction of inoculum and disease incidence through the use of one or more organisms other than humans (Cook and Baker, 1983). The National Academy of Science (1987) defines biological control as the use of natural or genetically modified organisms to reduce the effects of disease causing organisms while favoring desirable organisms (plant life, animals, and beneficial insects and microorganisms). DeBach (1964) has a more specific concept that includes the use of parasites, predators, and pathogens to maintain another organism's (in this case a plant pathogen) population at a lower level than would occur in the absence of those controlling organisms. Gabriel and Cook (1990) advocate the broader definition such as that by the National Academy of Science and further

contend that disease control should be divided into physical, biological, and chemical control. In this definition, biological control is the use of natural or genetically modified organisms to act as antagonist to a pathogen. Included in this definition is host resistance; either natural or genetically manipulated. However, Gabriel and Cook (1990) point out that cultural methods as well as conventional plant breeding can be a part of biological control. Biological control, as defined, largely depends on bacterial or fungal antagonist or other microorganism derived metabolites that suppress or otherwise inhibit disease development. While research looking at these processes has been fairly successful in sterile soils or other controlled situations, less success has occurred in non-sterile fields (Baker, 1978; Malajczuk, 1983). For instance, many bacteria and fungi are antagonistic to *Phytophthora*, but effectiveness in the field is limited. One successful system is a field or cropping situation is the so-called "Ashburner System" (Baker, 1978). This system relies on a cover crop rotation of various species to maintain a healthy soil microorganism population antagonistic to plant pathogens.

Resistance has been defined as the ability of a host plant to hinder the development of a pathogen (Robinson, 1969, 1976). Host resistance is the most important aspect of disease control since a resistant plant reduces or eliminates the need for fungicides or other plant protection chemicals. The goal of breeding or selecting resistant plants is to obtain a high level of resistance to disease, but keeping the required agronomic, horticultural, or other attributes.

As with biological control, many concepts of resistance exist, especially in the terminology used to describe resistance. Umaerus and others (1983) suggested the terms specific resistance and general resistance. Others contend that the term general resistance is vague and should not be used (Robinson, 1969). Muller (1953) referred to host specific resistance as true resistance and is also known as vertical resistance (Vanderplank, 1963). The term general resistance as defined is identical to the term horizontal resistance (Umaerus et al., 1983; Thurston, 1971). General resistance is also known as field resistance (Wastie, 1991). The term tolerance as suggested by Schimitthenner and Walker (1979) may be more descriptive and understood more clearly by producers and growers. Their definition of tolerance was the ability of a susceptible plant (soybean) to endure infection by *Phytophthora sojae* Kaufmann and Gerdemann

without severe symptoms while resistance means no infection. Physiologic or pathologic race are terms to describe microorganisms that are morphologically identical, but differ in cultural requirements, or physiological and biochemical traits (Hawksworth et al., 1983). This term implies that a particular biotype or "race" is pathogenic on specific plant genotypes (cultivars, botanical varieties), but not pathogenic on other plants of the same species.

Race-specific resistance or vertical resistance is characterized by single-gene interactions between the host and the pathogen. Host plants tend to be resistant to a specific pathological race (Erwin and Ribeiro, 1996). Race-specific resistance reduces the pathogenic inoculum to almost zero or very low levels since the initial infection does not spread beyond a few cells on the leaf surface (at least in leaf blights of potato caused by *Phytopthora infestans*) and cannot produce subsequent sporangia. Since race-specific resistance prevents an increase in inoculum, it acts as a sort of sanitation method (Vanderplank, 1984) The major drawback to race-specific resistance is readily apparent in that pathogenic organisms continually mutate creating new races. These new races may have the ability to infect host plants that lack the single-gene resistance for the newly mutated race. Hence, the new race of pathogens can quickly multiply negating the resistance of the original race-specific plant host (Vanderplank, 1971).

General or horizontal resistance usually involves multiple gene expression. The resistance is known as rate-limiting in that many components make up the resistance mechanisms. The rate of infection is much slower than in a non-resistant plant. This reduction in rate may slow the infection of certain plant parts until environmental conditions (loss of free water, lower humidity, optimal temperatures) preclude further disease development (MacKenzie et al., 1983; Parlevliet, 1979). General resistance is considered to be more stable than race-specific resistance. The supposition is that two or more chance mutations occurring simultaneously in the pathogen are rare (Vanderplank, 1971, Wastie, 1991).

As alluded to throughout this review, mechanisms to reduce the incidence of disease such as cultural, biological, or resistance seldom offer complete eradication or even control of diseases caused by *Phytophthora*. Chemical control is usually a necessary component in an overall control strategy. Many different chemicals with

varying modes of action are included in this control arsenal. Fungicides are chemical formulations that either destroys a fungus or inhibits or suppresses growth. Fungistats similarly suppress sporulation and growth, but do not actually kill the fungus. While most modern chemicals are actually fungistatic, the general term fungicide is most commonly used (Smith, 1980). Two broad modes of action are used to chemically control *Phytophthora*. The first is the eradication of inoculum by biocides or chemicals which kill propagules of *Phytophthora*. These types of chemicals kill all types of living organisms and as such must be used with extreme care around desirable plants. Biocides are normally used to treat such items as pots, benches, growing substrates, and in some cases irrigation water (Erwn and Ribeiro, 1996). The second broad mode of control are plant protectant fungicides which can be applied and act topically on the plant surface or systemically. These can act as inoculum eradicants, protectants, or as curative (Erwin and Ribeiro, 1996).

Commonly used biocides include many chemicals used in everyday life. An effective biocide with multiple uses is sodium hypochlorite, the active ingredient in household chlorine bleach. A 10 % solution is highly effective in treating exposed surfaces and tools. It is also effective in surface sterilization of seeds and other plant parts. However, since sodium hypochlorite is a strong oxidizer, it is very corrosive to susceptible equipment, has a short residual time, and must be frequently re-applied (Erwin and Ribeiro, 1996). Other biocides include the use of chlorine or other halogen gases to treat irrigation water similar to those systems used to treat public water supplies. The most common method of treating any water supply (either potable or industrial wastewater) including nursery irrigation water is the injection of chlorine or bromine, as chlorine or bromine gas, into the water stream (Ferraro and Brenner, 1997). Treatment concentrations vary widely depending on types of influent or effluent being treated. Normal domestic wastewater effluents require a range from 1 to 3 $mg \cdot L^{-1}$ residual free chlorine to meet most state and federal standards (De Hayr et al., 1994; Grasso, 1996). Water sources high in organic and inorganic contaminants may require as much as 25 to 30 $mg \cdot L^{-1}$ chlorine to achieve the required 1 to 3 $mg \cdot L^{-1}$ residual chlorine due to chemical binding (De Hayr et al., 1994). The major factor for the use of chlorination in water treatment in horticultural operations is that low to moderate levels (>7 $mg \cdot L^{-1}$) of chlorine

may be lethal or phytotoxic to many plant species (Ferraro and Brenner, 1997). In addition to negative plant growth responses, chlorine can be very corrosive to equipment, may form undesirable secondary organic by-products such as trihalomethane, and can be an explosive and health hazard (Grasso, 1996). When chlorine, the biocidal element, is used to treat irrigation water, dosages must be adjusted to achieve effective control but not phytotoxicity.

Copper naphthenate is effective in suppressing inoculum on wooden benches used in greenhouse or nursery operations. A periodic routine re-application of the chemical is effective to suppress residual inoculum in cracks and seams in benches. Copper napthaenate is toxic to plant roots if in direct contact (Baker, 1957). Other copper containing compounds have been shown to kill mycelium of *Phytophthora* (Smith, 1979).

Quaternary ammonium compounds have been used to eradicate species of *Phytophthora* such as *Phytophthora cinnamomi*. The quaternary ammonium compounds were found to be more effective than phenolic compounds (Noske and Shearer, 1985). This type of chemical is also useful for treating containers, tools, and other equipment.

Two chemicals traditionally used as soil sterilants or fumigants are methyl bromide/chloropicrin formulations and isothiocyanate (metham sodium)(Smith, 1980). These two chemicals are highly effective as general biocides including nematicidal action (Erwin and Ribeiro, 1996). These chemicals are expensive to apply and require specialized equipment and handling to use. Adverse environmental impacts and public concerns have severely restricted their use to small areas of intense production of high value crops (Erwin and Ribeiro, 1996).

Fungicides usually are classified as eradicative, protective, or curative. Many of the modern plant protection chemicals are systemic (taken up by the plant and translocated) and may be multifunctional and therefore lacking a discreet mode of action i.e., they may be both protective and curative. This multiplicity may vary by dosage and other situations (Heitefuss, 1989). Protectant fungicides are applied directly to the plant, usually as a spray coating the foliage. This differs from eradicant fungicides (such as methyl-bromide) which are used to sterilize equipment or act as pre-plant soil sterilants. Protectant fungicides often have a metallic cation component which many times are copper, tin, zinc, or manganese based (Schwinn and Margot, 1991). These are usually

coupled with various dithiocarbamate formulations. Other protectant chemicals include chlorthalonil and phthalimide compounds. These chemicals exert a protective effect, but are not translocated in the plant and must be re-applied (Schwinn and Margot, 1991)

Systemic fungicides are chemical formulations that are taken up actively or passively through several plant structures (leaves, stems, roots, flowers) and translocated or moved from place of absorption to another area of the plant. Transport can be translaminar (across the leaf), apoplastically (upwards towards the meristem), or symplastically (downward movement)(Erwin and Ribeiro, 1996). Many systemic fungicides move upwards via the transpiration stream, while other fungicides can move downward via solute flow in the phloem (Erwin and Ribeiro, 1996). The effectiveness of systemic fungicides are greater than protectant fungicides in that they are less likely to wash off during rainfall or irrigation events. Additionally, they can suppress a pathogen after infection takes place. Some fungicides can act as a curative if disease pressure is not too high (Platt, 1985). *Phytophthora* may be controlled through many classes of systemic chemicals. These chemical classes include the carbamates, isoxazoles, cyanoacetamide oximes, ethyl-phosphates, and phenylamides (Erwin and Ribeiro, 1996). As with any fungicides, these chemicals should be used carefully and as part of an overall control strategy to mitigate the chances of *Phytophthora* developing fungicide resistant races.

Novel Methods of Control in Irrigation Supplies

An alternative method of treating water supplies is the use of radiant energy in the form of ultraviolet radiation (UV) excimer lamps emitting radiation at 172 and 222 nm (Ramsay et. al, 2000). The use of ultraviolet radiation in a disinfection procedure works by inducing photobiochemical changes within a microorganism. Two criteria must be met in order for UV light to be effective; namely, the radiation must be of sufficient energy to alter chemical bonds, and the radiation must be adsorbed by the organism (Grasso, 1996). The ability to deliver radiation from the UV generating source to the target organism is crucial to the performance of UV disinfection systems. The major problem in UV disinfecting systems in horticultural production facilities is dissolved and fine particulate matter causing turbidity in the water to be treated (Grasso, 1996). These materials may impede or absorb UV radiation transmission through the liquid being

treated. Limitations on radiation delivery can also be caused by the deposition of insoluble materials, such as various mineral salts, on the surface of the quartz jackets that typically surround and house the UV source lamps (Grasso, 1996), resulting in a process known as fouling. Hard water or water high in ferric (Fe^{3+}) iron oxides may also result in fouling of the lamp surfaces (Grasso, 1996). Another disadvantage to using UV radiation is a phenomenon known as photoreactivation and dark repair in which microorganisms have evolved biological systems to repair damage by sub-lethal exposure to disinfectants such as UV light (Grasso, 1996) This phenomenon can be overcome by treating the water with successive UV light exposures followed by a dark period. Such systems are costly to build, maintain, and operate and are not usually feasible for most horticultural operations. Other water treatment strategies include heat, oxidizing chemicals (hydrogen peroxide), and membrane filters (Ehret et al., 2001).

Ozone gas has been used to treat municipal drinking and industrial waste water. Ozone has been used in municipal water treatment facilities since the late 1800's (Vosmaer, 1916). To date, little research has been published on using ozone to treat irrigation water in horticultural production operations for control of *Phytophthora* (Ferraro and Brenner, 1997). Studies in The Netherlands looking at recycled water in greenhouse hydroponic production systems have shown that recycled water can save up to 30% of the water used and up to 40% of fertilizer usage (Runia, 1994). However, the possibility of plant pathogens infesting the irrigation water that is being recycled is a real concern (van Os, 1999). Further studies in The Netherlands on closed irrigation systems (captured and recycled hydroponic systems) show ozone to be effective in reducing microorganisms in drainwater (Runia, 1994, 1995). However, these studies dealt with relatively small amounts of water (<1000 L) used in soilless substrate (rockwool) under greenhouse conditions. There are reported cases where a few nurseries on the West Coast of the United States have used ozone generators for disinfection purposes, but data on efficacy has not been published and industry practices and procedures tend to remain proprietary (Reeser, 1998).

Research in the food processing industry has examined using pressure and turbulence to eliminate bacteria and fungi during certain processes (Hoover, 1992). This methodology usually requires a pressure chamber and is not economical for treating large

volumes of irrigation water. Surfactants have been shown to lyse zoospores of *Phytophthora* but not mycelium or encysted zoospores (Stranghellini and Tomlinson, 1987; Stranghellini et al., 1996). If surfactant levels can be maintained in the irrigation stream, plants are protected from zoosporic inoculum but not from other forms of inoculum (Moorman and Lease, 1999). Additionally, the surfactant must not be phytotoxic. Antimicrobial substances containing copper and zinc (Smith, 1979; Tomlinson and Faithfull, 1979; Toppe and Thinggard, 1998), hydrogen peroxide, sodium phosphate, phosphorus acid, and EDTA (ethylene diamine-tetraacetic acid: a chelating agent) have been used to control pathogens in water supplies (Fett, 2002; Runia, 1995; Yun, 2003). Calcium has been added to irrigation water to disrupt the motility of zoospores of *Phytophthora*. While not a completely effective biocide, calcium may play a role in reducing inoculum (Deacon and Donaldson, 1993; Reid, et al., 1995). All of the above listed non-chemical or non-traditional methods of treating water have advantages and disadvantages and must be carefully evaluated in terms of cost and benefits.

The purpose of the following studies are to investigate the efficacy of ozone gas in controlling *Phytophthora capsici* in horticultural irrigation water as part of an overall strategy to reduce the incidence of plant disease from pathogens in recirculated irrigation water. Studies include the role of turbid water in ozone efficacy, the pathogenicity of the selected plant pathogen, and possible phytotoxic effects of ozone on vegetative growth. Additional studies look at ozone interactions with a commercially used water soluble fertilizer, and a preliminary study looking at possible inactivation of a plant growth regulator using ozone gas.

CHAPTER V
OZONE EFFICACY ON *PHYTOPHTHORA CAPSICI*

Phytophthora capsici Leonian is a virulent plant pathogen that attacks a large number of plant species and has been isolated in recirculated irrigation water in addition to causing disease and was chosen as a model organism to represent a pathogen encountered in horticultural production. Ozone gas introduced into water has powerful oxidizing capabilities and will control a large number of microorganisms including bacteria, fungi, viruses, and protozoa-like organisms (Rice and Browning, 1981; Rivas et al., 2001; Runia, 1994). Ozone is often effective in low concentrations in the range of 0.1 to 5 mg· L^{-1}(Flusche, 2006). Ozone injection has been used for a variety of commercial purposes including control of pathogens in municipal water supplies (Rice et al., 1986), but has not been experimentally tested for use on recirculated nursery or greenhouse water to control *Phytophthora.* Reeser (1998) states that nurseries in the state of Oregon use ozone in treating irrigation water, but does not present information on ozone concentrations or methodology. Information concerning specific commercial operations is often considered proprietary and data remains unpublished. In a review of plant pathogens in irrigation water, Hong and Moorman (2005) state that a lack of published reports of pathogen control strategies under actual operating conditions constitutes a serious gap in knowledge. The purpose of this study was to determine the efficacy of ozone gas in controlling a species of *Phytophthora* in horticultural irrigation water.

Materials and Methods

A pure isolate of *Phytophthora capsici* Leonian (1922) was obtained from American Type Culture Collection (ATCC), Manassas, Va. (ATCC Accession number 66630). This culture was originally isolated from commercially produced chili peppers *Capsicum annuum* L. grown in New Mexico, U.S. To obtain this particular isolate from ATCC, it was necessary to obtain a plant protection and quarantine permit from the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS PPQ 526 Permit) countersigned by the Texas Department of Agriculture. This permit dictates pathogen handling, transport, and disposal requirements and laboratory conditions. The laboratory where this work was conducted was classified as a

Center for Disease Control CDC Level I lab. *Phytophthora capsici* was chosen because of its virulence and ability to infect a large number of plant species. The ATCC maintains a limited amount of readily available material and obtaining specific cultures involves significant lead time. *Phytophthora capsici* was in stock at the time of choosing an organism.

Culture media was a modified vegetable juice agar based on that described by Miller (1955). A 0.163 L aliquot of vegetable juice (V8, Campbell Soup Co., Camden, N.J.) was poured into in a 2 L heat resistant borosilicate flask and diluted with distilled water to an initial volume of 0.5 L. The solution was amended with 2 g of $CaCO_3$ (Producers Coop., Bryan, Texas), and was dissolved by stirring. Agar (15 g) (Fisher Granulated Agar, Fisher Scientific, Fair Lawn, N.J.) was added and the final volume brought to 1L with distilled water and placed on a stir plate to insure mixing. Final pH was adjusted to pH 7.0 with 0.1 N H_2SO_4. The flask opening was covered with aluminum foil and steamed autoclaved for 15 min. at 110 kPa and 115° C. After autoclaving, agar media was dispensed into either 4.5 or 6.5 mm plastic culture plates (Fisher Scientific, Fair Lawn, N.J.) under a laminar flow transfer hood using aseptic techniques. The 6.5 cm culture plates were used for culture maintenance while the 4.5 cm culture plates were used for regrowth plating so as to accommodate more plates under a restricted amount of bench space under lights. After agar media set, culture plate lids were sealed with a paraffin sealing film (Parafilm "M", American Can Co., Greenwich, Conn.).

From the original ATCC culture tube, 25 mm^2 (5 mm x 5 mm) sections of the agar containing mycelium of *Phytophthora capsici* were excised and transferred to the surface of fresh culture plates containing the above agar media using sterile techniques under a laminar flow transfer hood. Plates were sealed with paraffin sealing film and placed on a work bench in the laboratory under a fluorescent light bank containing two tubes emitting an average 35 $\mu mole \cdot m^{-2} \cdot s^{-1}$ PAR. Ambient room temperature was 22 C ± 2° C. Twenty culture dishes were re-plated at 2 to 3 week intervals to maintain viable cultures and to supply a suitable number of plates for specific studies.

Mycelium from maintenance cultures were transferred to fresh culture plates and were grown as above for 1 week. At this time, mycelium covered the plates. Plates were

rinsed five times at 30 min. intervals with 10 ml autoclaved distilled water at ambient temperature (20 ± 2° C). Water was decanted after each washing. Plates were stored in the dark at ambient temperature for a minimum of 24 h. Plates were then held at 5° C for 1 h and then returned to the bench under growing conditions listed above. Plates were observed under a microscope for sporangium development which usually began within 24 h. Sufficient culture plates were grown to have ample material for each subsequent study. All activities pertaining to preparation of samples to be treated were done using aseptic techniques under a laminar flow transfer hood. Upon completion of each study, all contaminated equipment and culture plates were autoclaved to eliminate the possibility of accidental release of propagules of *Phytophthora capsici* organism into the general environment and to fulfill USDA permit requirements.

Sporangium along with associated mycelia fragments were serially diluted by excising a 1 cm^{-2} piece of the culture and transferring to a sterile 50 ml capacity conical bottom plastic tube with screw tight cap (BD Falcon, Fisher Scientific, Hampton, N.H.). Fifty ml of previously autoclaved distilled water was added to the tube and gently agitated to disperse the organisms. A 1 ml sample of this tube was then pipetted into another conical tube and 50 ml of sterile water was added as previously described and gently agitated. Fifty ml of water allowed sufficient volume for subsequent ozonation. Excess agitation was avoided to reduce possible encystment of spores. At this point, a pipette was used to place a sample on a hemacytometer with Neubauer markings and a maximum volume of 0.1µ L (Cole Parmer Instruments, Vernon Hills, Ill.) and placed under a light compound microscope (Binocular Compound Microscope, Carl Zeiss Inc., Thornwood, N.Y.) at 50 to 100 X magnification for quantification. Number of spores per ml of sample was calculated by counting spores within the Neubauer markings multiplied by a dilution factor (50 ml) and multiplied by 10,000. Dilution continued until spore count of about 1 x $10^5 \cdot ml^{-1}$ was obtained. This value was chosen as it is a level commonly used in disinfection studies conducted with *Escherichia coli* T. Escherich, a common fecal coliform group of bacteria often found in water supplies (Haas and Finch, 2001). Once an appropriate dilution was obtained, replicate tubes were assembled under aseptic conditions. The conical tubes were modified by punching a hole with a heated cork borer in the top of the screw on cap with a diameter large enough to admit the ozone

generator gas feed line (6.5 mm o.d.). The hole was subsequently sealed with paraffin film. The caps were modified to reduce the possibility of microbial contamination by minimizing the open surface area exposed during the actual ozonation process.

To reduce possible cross contamination, prepared sealed tubes were surface sprayed with 90% ethyl alcohol (EtOH), placed in a foam tube carrier, and placed in a sealable plastic bag also sprayed with 90% EtOH for transportation to the ozone generator locale which was not under aseptic conditions. Prior to treatment, ozone generator parameters were established to achieve the desired ozonation concentrations at the 50 ml volume. Ozone concentration was determined by the indigo colorimetric method. This procedure is based on the rapid and stoichiometrical decolorization of indigo trisulfonate (from indigo blue to clear) when in the presence of ozone (Bader and Hoigne, 1986). The source of the indigo trisulfonate was AccuVac ozone reagent low (0-0.2 mg·L-1) and high range (0.25-1.0 mg· L^{-1}) evacuated ampuls (Hach Co., Loveland, Colo.). The tip of an ampul containing a measured amount of reagent is inserted into a sample of the ozonated water and crushed. The vacuum within the ampul draws in a 15 ml amount of the ozonated liquid. The ampul is inverted twice to insure mixing and then immediately placed in a portable ozone meter (Hach Co., Loveland, Colo.) and the ozone concentration is read directly as $mg \cdot L^{-1}$ O_3. For concentrations greater than 1.0 $mg \cdot L^{-1}$ O_3, a color comparison wheel (Hach Co., Loveland, Colo.) was used to determine O_3 concentrations up to 1.5 $mg \cdot L^{-1}$. Ozone gas source was from an experimental catalytic electrochemical stack generator with a rated output of 0.113 kg· d^{-1} (Lynntech Industries, College Station, Texas). Ozone generator operating parameters were set at 9 amp output with an ozone flow rate of 5 $ml \cdot L^{-1} \cdot s^{-1}$. Ozone levels increase to a peak level and then ozone decay occurs either through ozone demand, or ozone degradation due to its inherent instability. Ozone treatments were 0, 0.5, 1.0, and 1.5 $mg \cdot L^{-1}$ at peak concentrations and were selected based on detectability limitations and probable effective dosages. Five prepared tubes containing an estimated 1 x $10^{5} \cdot ml^{-1}$ spores were used per ozone concentration (4 O_3 concentrations x 5 replicates = 20 tubes total). Each tube was removed from the plastic carrier bag and the ozone gas feed line was inserted into the hole after peeling back the paraffin film. After the required treatment time to reach peak ozone concentration (0, 30, 60, or 120 s), the film was replaced to seal the hole, the tube

was once again surface sprayed with 90% EtOH and returned to the carrier bag. Each subsequent tube was treated in a like manner. Tubes were allowed to stand for 1 h after treatment to allow for ozone diffusion through the sample and for transport back to the laboratory.

After ozonation, tubes were returned to the laboratory and placed under aseptic conditions. A sample was pipetted from each tube and observed under the hemacytometer as before for spore survival quantification. Prepared 4.5 mm plastic culture plates of fresh media containing the vegetative juice substrate were flooded with 1 ml of liquid pipetted from each tube and replicated 3 times for a total of 60 plates (4 O_3 concentrations x 5 replicates per O_3 concentration x 3 replicates per re-growth plate = 60 plates total) and randomly arranged on a laboratory bench under fluorescent lights emitting 35 μmole· m^{-2}· s^{-1} PAR at ambient room temperature (22 ± 2° C). Plates were observed at 12 h intervals for 36 h for signs of re-growth. New colony forming units per ml of sample (CFU·ml^{-1}) were counted and recorded using a binocular dissecting microscope (SMZ1, Nikon Inc., Tokyo, Japan). Rapid mycelial growth precluded counting individual colony formation much after 24 h of first observed re-growth. New mycelial growth was then observed under light microscopy for comparison with original cultures. The experimental design of the re-growth phase was a complete factorial randomized design. The experiment was initially started on 7 November, repeated on 14 November, and again repeated on 5 December, 2006 for a total of 3 separate treatment dates. Possible interactions between ozone concentration and treatment date and main effects of ozone concentration and treatment date by each observed time on CFU·ml^{-1} were analyzed using SAS PROC GLM and means comparison were made using least squares means procedures (SAS 9.1 for Windows, Institute, Cary, N.C.).

Results and Discussion

The ozone production, or gas evolution, from the generator was uniform and mass transfer in reverse osmosis water was predictable and reproducible with several initial operating tests giving consistant results in reaching desired ozone concentrations for all subsequent experiments (Fig. 1). Ozone decay in the irrigation water treatments followed a typical ozone mass transfer reaction whereby an initial lag time occurs in increasing ozone concentration as ozone dissolves and diffuses through the water sample (Fig.2).

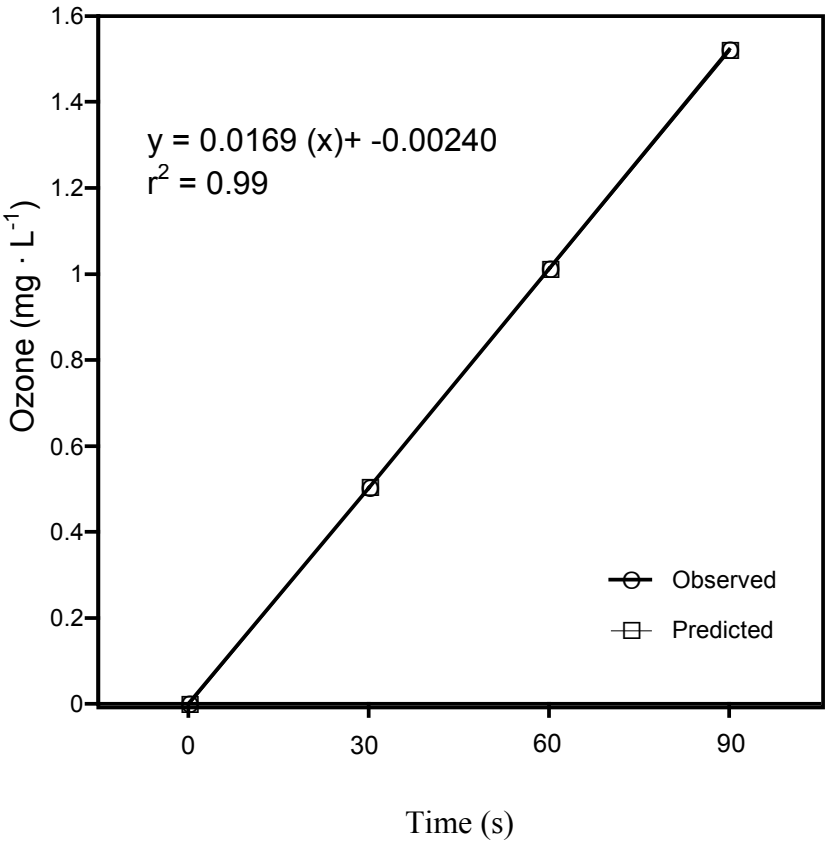

Figure 1. Linear regressions ($P< 0.001$) of observed and predicted values of ozone production over time (min.) from a electrochemical stack cell generator. Symbols for the means ± standard error bars are partially obscured by predicted symbols. Means represent observations of 5 replications.

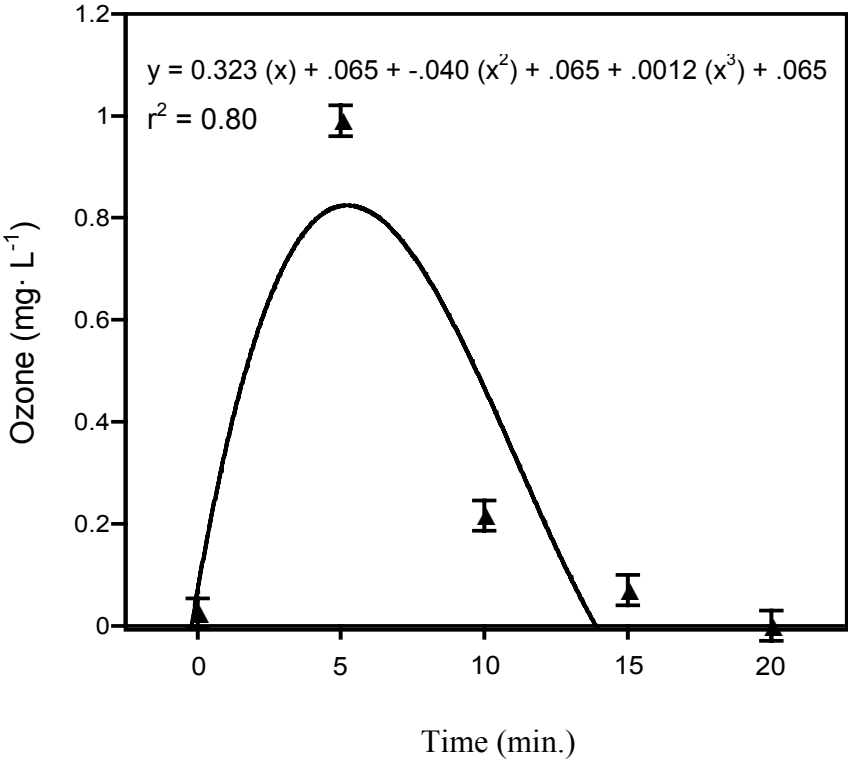

Figure 2. Predicted regression curve ($P< 0.001$) and observed values of ozone decay over time (min.) in reverse osmosis water using the electrochemical stack cell generator. Verticle bars represent standard errors of means of 5 observations.

The number of colony forming units per ml of sample ($CFU \cdot ml^{-1}$) observed growing at 12 and 24 h after inoculating agar plates with ozone treated spores of *Phytophthora capsici* over three dates indicates a significant reduction in $CFU \cdot ml^{-1}$ as ozone concentration increased from 0 to 1.5 $mg \cdot L^{-1}$ O_3 (Fig. 3). Across all treatment dates (7 Nov., 14 Nov., 5 Dec.), a 12 h incubation period shows a reduction in $CFU \cdot ml^{-1}$ from 5.2 $CFU \cdot ml^{-1}$ at 0 $mg \cdot L^{-1}$ to 4.2 $CFU \cdot ml^{-1}$ at 0.5 $mg \cdot L^{-1}$ O_3. At ozone concentrations of 1.0 $mg \cdot L^{-1}$ O_3, $CFU \cdot ml^{-1}$ are reduced to 0.4 or less than 1 $CFU \cdot ml^{-1}$ per plate at 12 h. A similar pattern emerges at 24 h after inoculation (Fig. 4). At 24 h, additional $CFU \cdot ml^{-1}$ are observed as more intact inoculum germinates and grows. At 0 $mg \cdot L^{-1}$ O_3 an average of 6.5 $CFU \cdot ml^{-1}$ formed across dates, an increase of 1.3 $CFU \cdot ml^{-1}$ over the 12 h incubation period. At 0.5 $mg \cdot L^{-1}$ O_3, 5.2 $CFU \cdot ml^{-1}$ formed with a decrease to 0.5 $CFU \cdot ml^{-1}$ at 1.0 $mg \cdot L^{-1}$ and 0 $CFU \cdot ml^{-1}$ at 1.5 $mg \cdot L^{-1}$ O_3. Colony forming units per ml significantly decreased at each observed time interval by treatment date (Figs. 3 and 4). The December treatment date had the lowest $CFU \cdot ml^{-1}$ across ozone concentration and observed time interval. This was attributable in part to improved protocol technique and manipulation of the samples over time. An additional factor could have been the physiological condition of the inoculum from different treatment dates. However, the trends were consistent and an ozone concentration of 1.5 $mg \cdot L^{-1}$ prevented $CFU \cdot ml^{-1}$ of *Phytophthora capsici* under the treatment conditions. Post-ozonation treatment observations of the aliquots containing spores using a hemacytometer showed spore and mycelial fragments still in the solution, but it was not possible to tell whether the structures were viable propagules or lyzsed cellular remains. Biological stains exist to observe intact spores, but stains such as a 0.1% aniline blue dye involve "fixing" the organism in lactophenol thereby affecting re-growth (Erwin and Ribeiro, 1996). Re-plating and subsequent reduced $CFU \cdot ml^{-1}$ formation with increasing ozone concentration is a better verification of ozonation efficacy than post-treatment spore quantification. As a comparison of ozone efficacy using other types of microorganisms, studies looking at reducing viruses and bacteria in water found that *Escherichia coli* was 99.99% inactivated at ozone concentrations of 0.038 $mg \cdot L^{-1}$ at a 30 s exposure (Boyce et al., 1981). A poliovirus (Sabin Type 1) was 97% inactivated at a concentration of 0.031 $mg \cdot L^{-1}$ O_3 after a 30 s exposure (Boyce et al., 1981). Hong and Moorman (2005) report

researchers in Japan showing differing sensitivities of root-infecting plant pathogens to ozonation in hydroponic solutions. These researchers (Yamamoto et al., 1990) looked at *Corynebacterium michiganense* (E.F Smith) Jensen, *Pseudomonas solanacearum* E.F Smith, *Erwinia carotovora* subsp. *carotovora* (Townsend) Holland, and *Fusarium oxysporum* Schlechtendahl f. sp. *lycopersici* (Saccardo) Holland, but not species of *Phytophthora*. This research from Japan ozonated a hydroponic test solution containing a complete nutrient solution and the various microorganisms at an ozone gas feed rate of 10 $L \cdot min^{-1}$ but did not report actual ozone concentrations in the nutrient solutions. They did report an immediate drop in viable cell numbers of the organisms after a 15 min. exposure with almost complete inactivation of the organisms after 60 min. exposure to ozone at the 10 $L \cdot min^{-1}$ input rate. No ozone concentrations in the nutrient solutions are given for the various time intervals. In developing the ozonation protocols, precautions were taken, such as conical tube modification and surface sterilization and transport, to insure that the *Phytophthora capsici* or the treated water samples were not contaminated by unknown micro-organisms during the various treatment phases. Results indicate that peak ozone concentrations in the range of 1.5 $mg \cdot L^{-1}$ applied to batch treatments of water is effective in controlling *Phytophthora capsici*. The 1.5 $mg \cdot L^{-1}$ O_3 concentration was greater than the reported rate of 0.5 to 1.0 $mg \cdot L^{-1}$ concentration reported as being effective for coliform bacteria control in drinking water treatment systems. One possible explanation for the increased ozone demand is that the glue bonding together the sand-based aeration stones used in these studies was attacked and degraded by the ozone during the course of treating the samples. Over a period of a few hours, the aeration stones were reduced to sand and this was attributed to the action of the ozone gas. Therefore, aeration stones were replaced before each treatment to insure uniformity and this may explain the higher ozone demand in these studies.

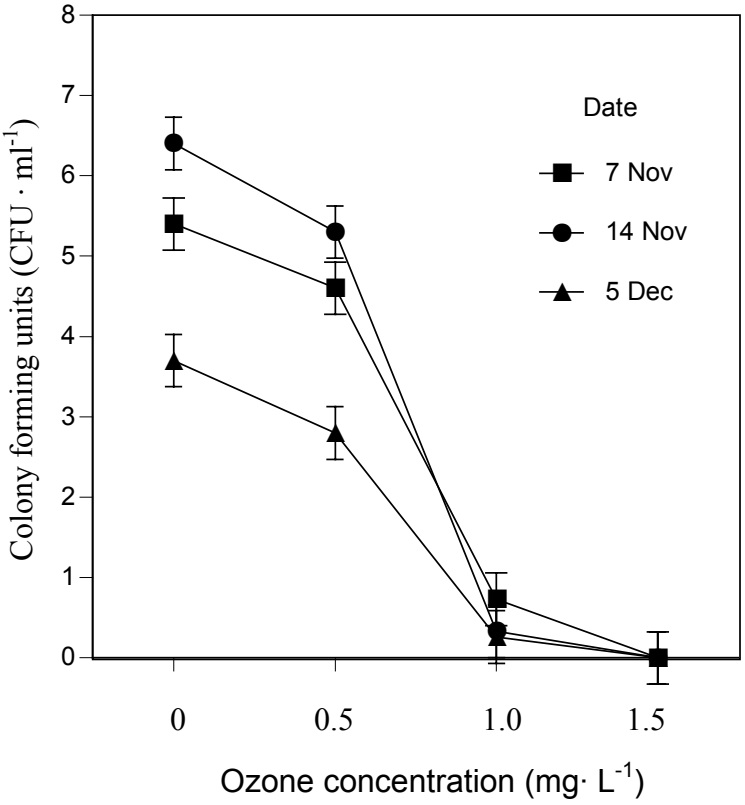

Figure 3. Colony forming units per ml of sample ($CFU \cdot ml^{-1}$) at 12 h after inoculation for the three treatment dates with 4 ozone concentrations. Symbols represent means of 15 observations and vertical bars represent standard errors. The treatment date by ozone concentration was significant at $P \leq 0.05$.

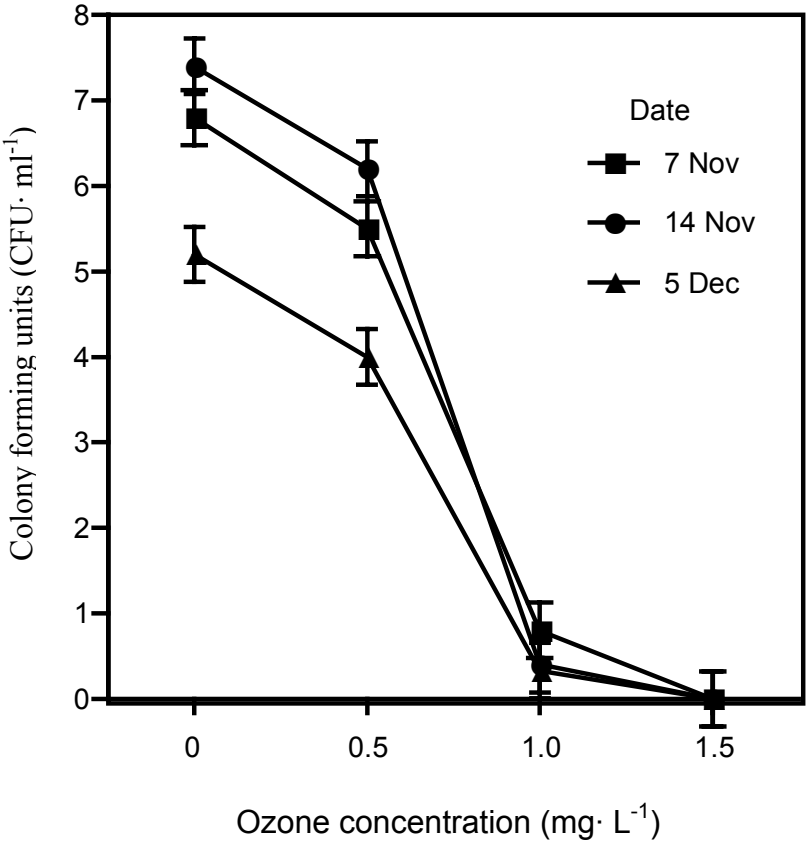

Figure 4. Colony forming units per ml of sample (CFU·ml^{-1}) at 24 h after inoculation for the three treatment dates with 4 ozone concentrations. Symbols represent means of 15 observations and vertical bars represent standard errors. The treatment date by ozone concentration interaction was significant at $P \leq 0.05$.

CHAPTER VI
EFFECT OF TURBID WATER ON EFFICACY

Irrigation runoff collection systems from greenhouse and nursery operations usually consists of canals, channels, swales, or other configurations that capture excess irrigation and rainfall and by gravity flow convey that water to a detention pond or other holding area for subsequent treatment or discharge. These collection basins may be lined with an impervious surface such as concrete, or may be unlined open surfaces. The possibility exists for the captured irrigation water to pick up particulate matter such as organic and inorganic growing substrates (i.e. peat moss, bark, sand, perlite, vermiculite) from greenhouse benches or floors and outdoor nursery bays along with soil or clay particles from the collection basins. This may be especially problematic during periods of heavy rainfall or in areas with soils that are highly subject to erosion. While much of this particulate matter may settle in holding tanks, or be removed by filtration, fine particulate matter such as suspended clay particles may escape settling and filtration and pass through to be re-applied in irrigation water. Microorganisms and viruses in particular have been found to be absorbed to clay particles or embedded in solid matter (Carlson et al., 1968; Schaub and Sagik, 1975). Microorganisms absorbed, embedded, or shielded by particulate matter may react differently or pass through disinfection systems in a viable state. Turbidity is a measure of the cloudiness of water and is measured in nephelometric turbidity units (NTU). The Interim Enhanced Surface Water Treatment Rule (IESWTR) recognizes the potential of pathogen protection by suspended particulate matter in water treatment facilities. The Environmental Protection Agency (EPA) sets limits on cloudiness in drinking water. Turbidity performance standards for surface water call for direct filtration combined filter effluent of $\leq$ 0.3 NTU in at least 95% of measurements taken each month. As of 2002, no turbidity greater than 1 NTU is allowed in surface derived drinking water (Environmental Protection Agency, 2001). Bentonite ($Al_2O_3 \cdot 4SiO_2 \cdot H_2O$) clay, a aluminum phyllosilicate, has been used to investigate turbidity in drinking water studies using ozone disinfection methods on bacteria and viruses (Boyce et al., 1981). Bentonite clay particles range in size from 3 to 8 μm, a size comparable to that found following treatment from a filtration system in water treatment

facilities (Tate et al., 1977). Previous studies have looked at a range of bentonite derived turbidity from 1 to 5 NTU based on older threshold contaminant levels of a 5 NTU maximum. Because of the likelihood of recycled horticultural irrigation water picking up sediments or other particulate matter during capture and reuse, the purpose of this study was to investigate the potential of *Phytophthora capsici* Leonian being absorbed to clay particles or otherwise shielded from ozone disinfection concentrations of 0.0, 0.5, 1.0, and 1.5 mg· L^{-1} O_3 using bentonite clay up to 2 NTU (2 times the EPA action level of 1 NTU) as a model for suspended clay particles in recycled irrigation water.

Materials and Methods

A pure isolate of *Phytophthora capsici* Leonian (1922) was obtained from American Type Culture Collection (ATCC), Manassas, Va. (ATCC Accession number 66630). This culture was originally isolated from commercially produced chili peppers *Capsicum annuum* L. grown in New Mexico, USA. To obtain this particular isolate from ATCC, it was necessary to obtain a plant protection and quarantine permit from the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS PPQ 526 Permit) countersigned by the Texas Department of Agriculture. This permit dictates pathogen handling, transport, and disposal requirements and laboratory conditions. The laboratory where this work was conducted was classified as a Center for Disease Control CDC Level I lab. *Phytophthora capsici* was chosen because of its virulence and ability to infect a large number of plant species. The ATCC maintains a limited amount of readily available material and obtaining specific cultures involves significant lead time. *Phytophthora capsici* was in stock at the time of choosing an organism.

Culture media was a modified vegetable juice agar based on that described by Miller (1955). A 0.163 L aliquot of vegetable juice (V8, Campbell Soup Co., Camden, N.J.) was poured into in a 2 L heat resistant borosilicate flask and diluted with distilled water to an initial volume of 0.5 L. The solution was amended with 2 g of $CaCO_3$ (Producers Coop., Bryan, Texas), and was dissolved by stirring. Agar (15 g) (Fisher Granulated Agar, Fisher Scientific, Fair Lawn, N.J.) was added and the final volume brought up to 1 L with distilled water and placed on a stir plate to insure mixing. Final pH was adjusted to 7.0 with 0.1N H_2SO_4. The flask opening was covered with aluminum

foil and steamed autoclaved for 15 min. at 110 kPa and 115° C. After autoclaving, agar media was dispensed into either 4.5 or 6.5 cm plastic culture plates (Fisher Scientific, Fair Lawn, N.J.) under a laminar flow transfer hood using aseptic techniques. The 6.5 cm culture plates were used to maintain the cultures over time while the 4.5 cm culture plates were used for subsequent regrowth plating to maximize limited lighted bench space. After agar media set, culture plate lids were sealed with a paraffin sealing film (Parafilm "M", American Can Company, Greenwich, Conn.).

From the original ATCC culture tube, 25 mm^2 (5 mm x 5 mm) sections of the agar containing mycelium of *Phytophthora capsici* were excised and transferred to the surface of fresh culture plates containing the above agar media using sterile techniques under a laminar flow transfer hood. Plates were sealed with paraffin sealing film and placed on a work bench in the lab under a fluorescent light bank containing two tubes emitting an average of 35 $\mu mole \cdot m^{-2} \cdot s^{-1}$ PAR. Ambient room temperature was 22 C ± 2° C. Twenty culture dishes were re-plated at 2 to 3 week intervals to maintain viable cultures and to supply a suitable number for of plates for specific studies.

Turbidity levels of 0.0, 0.5, 1.0, 1.5, and 2.0 NTU were determined to correspond with 0.0, 3.5, 7.0, 10.5, or 14.0 $mg \cdot L^{-1}$ bentonite clay (Sigma-Aldrich Chemical, St. Louis, Mo.), respectively. Peak ozone concentrations were 0.0, 0.5, 1.0, and 1.5 $mg \cdot L^{-1}$ O_3. Ozone concentration was determined by the indigo colorimetric method. This procedure is based on the rapid and stoichiometrical decolorization of indigo trisulfonate (from indigo blue to clear) when in the presence of ozone (Bader and Hoigne, 1986). The source of the indigo trisulfonate was AccuVac ozone reagent low (0-0.2 $mg \cdot L^{-1}$) and high range (0.25-1.0 $mg \cdot L^{-1}$) evacuated ampuls (Hach Co., Loveland Colo.). The tip of an ampul containing a measured amount of reagent is inserted into a sample of the ozonated water and crushed. The vacuum within the ampul draws in a 15 ml amount of the ozonated liquid. The ampul is inverted twice to insure mixing and then immediately placed in a portable ozone meter (Hach Co., Loveland, Colo.) and the ozone concentration is read directly as $mg \cdot L^{-1}$ O_3. For concentrations greater than 1.0 $mg \cdot L^{-1}$ O_3, a color comparison wheel (Hach Co., Loveland, Colo.) was used to determine O_3 concentrations up to 1.5 $mg \cdot L^{-1}$. Ozone gas source was from an experimental catalytic electrochemical stack generator with a rated output of 0.113 $kg \cdot d^{-1}$ (Lynntech Industries,

College Station, Texas). Ozone generator operating parameters were set at 9 amp output with an ozone flow rate of 5 $ml \cdot L^{-1} \cdot s^{-1}$.

Sporangium along with associated mycelia fragments were serially diluted by excising a 1 cm^{-2} piece of agar and transferring to a sterile 50 ml capacity conical bottom plastic tube with screw tight cap (BD Falcon, Fisher Scientific., Hampton, N.H.). Fifty ml of autoclaved distilled water was added to the tube and gently agitated to disperse the organisms. A 1 ml sample of this tube was then pipetted into another conical tube and 50 ml of sterile water was added and gently agitated. Fifty ml of water allowed sufficient volume for subsequent ozonation. Excess agitation was avoided to reduce possible encystment of spores. At this point, a pipette was used to place a sample on a hemacytometer with Neubauer markings and a maximum volume of 0.1µ L (Cole Parmer Instruments, Vernon Hills, Ill.) and placed under a light compound microscope (Binocular Compound Microscope, Carl Zeiss Inc., Thornwood, N.Y.) at 50 to 100 X magnification for quantification. Number of spores per ml of sample was calculated by counting spores within the Neubauer markings multiplied by a dilution factor (50 ml) and multiplied by 10,000. Dilution continued until spore count of about 1 x $10^5 \cdot ml^{-1}$ was obtained. Dilution factors varied greatly due to the status of the particular culture at the time of sampling and had to be determined for each set of experiments. The value of 1 x $10^5 \cdot ml^{-1}$ was chosen as it is a level commonly used in disinfection studies conducted with *Escherichia coli* T. Escherich, a common fecal coliform bacteria often found in water supplies (Haas and Finch, 2001).

A 5 x 4 complete factorial design with 5 replications per treatment combination (5 bentonite clay concentrations x 4 O_3 concentrations x 5 replications per treatment = 100 total) was established using 50 ml conical bottom tubes as previously described. Solutions containing 0, 0.5, 1.0, 1.5, or 2.0 NTU of bentonite clay suspended in distilled water were autoclaved at 120° C at 110 kPa for 15 min., cooled and then dispensed into 50 ml aliquots per bentonite treatment under aseptic conditions. Spores of *Phytophthora capsici* previously diluted to 1 x 10^5 ml were added (1 ml spore suspension) to the tubes containing the bentonite clay. Each tube cap had previously been modified by using a heated cork borer to make a hole (6.5 mm o.d.) into which the ozone gas feed line was inserted. This modification was to reduce the possible incidence of cross contamination

during the ozonation process. The tube caps were tightened and the top access hole sealed with paraffin film (Parafilm "M", American Can Co., Greenwich, Conn.) All transfers were under aseptic conditions in a laminar flow hood. Tubes were then surface sprayed with 90 % ethyl alcohol (EtOH) to reduce possible contamination and placed in foam holders and placed inside a sealed plastic bag used to transport the prepared samples to the ozone generator. Samples were ozonated to obtain peak concentrations of 0, 0.5, 1.0, 1.5 mg· L^{-1} O_3. The paraffin film was replaced after each ozonation treatment and each tube was surface sprayed with 90% EtOH prior to being returned to the foam carrier and placed in the plastic bag for transport back to the laboratory.

After ozonation, tubes were returned to the laboratory and placed under aseptic conditions. A sample was pipetted from each tube and observed under the hemacytometer as before for spore survival quantification. Each tube with a ozonated spore suspension was considered an experimental unit. Prepared 4.5 cm, to conserve bench space, plastic culture plates of fresh media containing the vegetative juice substrate were flooded with 1 ml of liquid pipetted from each tube and replicated 3 times for a total of 300 plates (5 bentonite clay concentrations x 4 O_3 concentrations x 5 replications per treatment x 3 replicates for replating = 300 total) and randomly placed on a laboratory bench under fluorescent lights emitting an average of 35 µmole· m^{-2}· s^{-1} at ambient room temperature (22 ± 2° C). Plates were observed at the end of 12 h for signs of re-growth and at 12 h intervals thereafter. At first sign of re-growth, new colony forming units per ml of sample (CFU·ml^{-1}) were counted and recorded. Rapid mycelial growth precluded counting colony formation much after 24 h of first observed re-growth. New mycelial growth was then observed under light microscopy for comparison with original cultures. Data were analyzed at each observation period for significance with NTU and ozone concentration as main effects and along with possible significant interactions between NTU and ozone concentrations using SAS PROC GLM and means were compared using least squares means procedures (SAS 9.1 for Windows, Institute, Cary, N.C.).

Results and Discussion

Regrowth of *Phytophthora capsici* in the form of CFU·ml^{-1} at 12 h and 24 h after inoculation of agar substrate plates was reduced as ozone concentration increased across all bentonite NTU levels (Figs. 5 and 6). At 12 h incubation, there was no significant ($P \leq$ 0.05) differences in the interaction between bentonite NTU and ozone concentrations. There was also no significant difference ($P \leq$ 0.05) in CFU·ml^{-1} and bentonite NTU concentration at 12 h incubation. However, there were significant differences in CFU·ml^{-1} among ozone concentrations at 12 h incubation (Fig. 5). Average regrowth ranged from 5.4, 4.0, 0.21, and 0 CFU·ml^{-1} across all NTU at 0.0, 0.5, 1.0, and 1.5 mg· L^{-1} ozone, respectively. At 24 h incubation, a similar trend developed where there was no significant ($P \leq$ 0.05) differences in the interaction between bentonite NTU and ozone concentration nor significant differences in CFU·ml^{-1} and bentonite NTU levels (Fig. 6). However, there was a significant difference in CFU·ml^{-1} at various ozone concentrations as in the 12 h incubation with regrowth means ranging from 6.6, 5.0, 0.28, and 0 CFU·ml^{-1} across all NTU at 0.0, 0.5, 1.0, and 1.5 mg· L^{-1} ozone, respectively. After 24 h incubation, rapid mycelial growth precluded identification of new CFU·ml^{-1}. These data indicate that under the test conditions bentonite clay at 0 to 2 NTU did not impede the ability of ozonated water samples to reduce the regrowth of *Phytophthora capsici* cultured *in vitro*. These data also re-affirm earlier tests investigating ozone efficacy in reducing *Phytophthora capsici* regrowth *in vitro* (Chapter V). Similar results have been reported for the relationship between turbidity and reduction of bacteria and viruses using ozonation with bentonite clay up to 5 NTU offering little or no protection to the organisms during the disinfection process (Boyce et al., 1981). Hoff (1978) reported that organic particulate matter better protected virus particles than bentonite clay. This report concluded that the type of matter (organic vs. inorganic) causing the turbidity is more important in providing protection than in the NTU level as particulate concentration. In horticultural operations, organic particulate matter such as peat moss, composted pine bark, and other substrate components and inorganic substrate components such as perlite, vermiculite, or sand, may be effectively removed from the recycled irrigation water via screens, settling ponds, or filtration systems. However, smaller suspended particulates such as clay are often harder to remove and may pass through filtration systems. These

data indicate that small inorganic particles such as bentonite clay (3 to 8 μM diameter) do not adversely affect the ozonation process in reducing propagules of *Phytophthora capsici* and subsequent reduced CFU when cultured *in vitro*. It should be noted that the tested water samples contained no organic matter which would present a greater demand for ozone. In field applications where recirculated water contains high amounts of organic matter or other contaminants from substrates or the production environment, the concentration of ozone needed to disinfect the water will be greater than the 1.5 $mg \cdot L^{-1}$ O_3 used in this study.

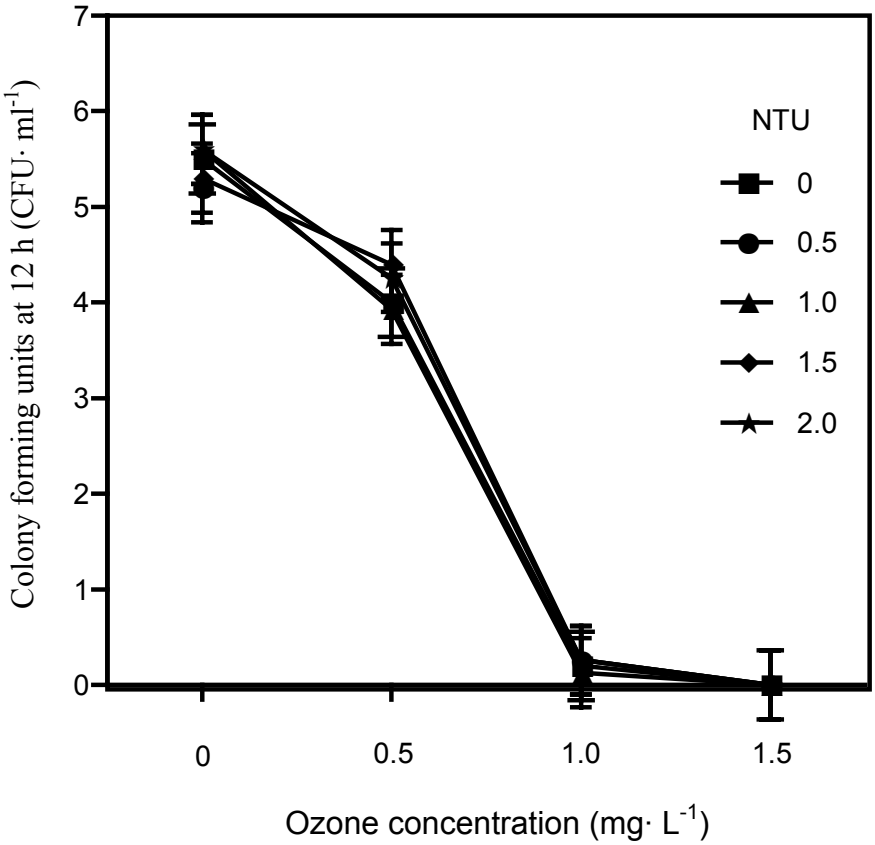

Figure 5. The effect of turbidity as nephelometric turbidity units (NTU) on colony forming units per ml of sample ($CFU \cdot ml^{-1}$) at 12 h incubation periods at concentrations of 0, 0.5, 1.0 , and 1.5 $mg \cdot L^{-1}$ ozone. Vertical bars represent standard errors. Values are means ± standard errors of 15 observations.

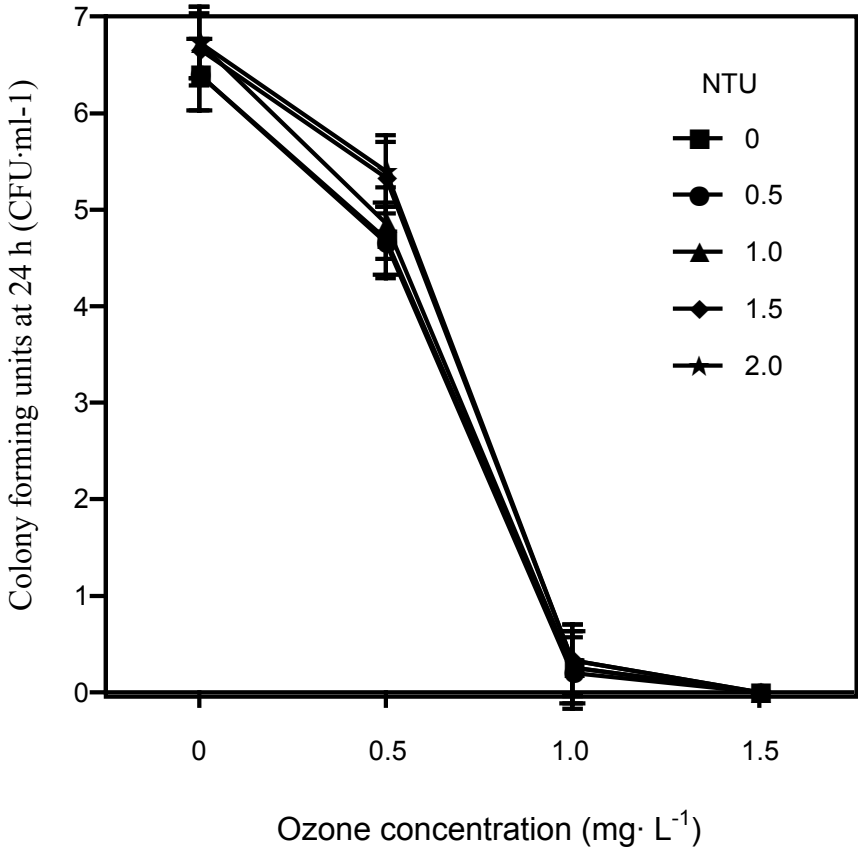

Figure 6. The effect of turbidity nephelometric turbidity units (NTU) on colony forming units per ml of sample ($CFU \cdot ml^{-1}$) at 24 h incubation periods at concentrations of 0, 0.5, 1.0, and 1.5 $mg \cdot L^{-1}$ ozone. Vertical bars represent standard errors. Values are means ± standard errors of 15 observations.

CHAPTER VII
BIOASSAY USING *CAPSICUM ANNUUM* SEEDLINGS

Plant pathogens grown *in vitro* or continuously sub-cultured on artificial substrate can lose virulence over time (Kelman, 1954). The term used to describe this loss of virulence is attenuation (Ibrahim et al., 2002) The causality of attenuated pathogens vary between differing types of pathogens (bacteria, fungi, viruses) (Ibrahim et al., 2002). Changes in polysaccharide production *in vitro* was correlated with virulence in *Erwinia amylovora* [(Burrill, 1882) Winslow et al., 1920], the causal agent of fire blight (Kelman, 1954). Loss of motility in virulent strains of *Pseudomonas solanacearum* Smith in broth culture allowed attenuated strains to proliferate since the non-motile virulent strains could not move and compete when oxygen became a limiting factor (Kelman, 1954). Researchers investigating pathogens infecting insects have found that changes in polysaccharide formation affects the ability of pathogen spores to adhere to insect surfaces affecting germination and invasion and hence virulence (Fargues, 1984; Morrow et al., 1989). The age of mycelium *in vitro* can affect sporangia production in some species of *Phytophthora* (Ribeiro, 1983). Juvenile mycelium depleted of nutrients produced more sporangia than older mycelium (Ayers and Zentmyer, 1971; Eye et al., 1978). One-day-old mycelium of *Phytophthora megasperma* f. sp. *glycina* Kuan and Erwin produced four times the sporangia as a five-day-old mycelium (Eye et al., 1978). *Phytophthora cinnamomi* Rands decreased the production of sporangia with increasing culture age when under axenic or pure culture conditions (Ayers and Zentmyer, 1971). In contrast, Gooding and Lucas (1959) found an increase in sporangium production by *Phytophthora nicotianae* Breda de Haan with increasing culture age.

To ensure the viability and virulence of the isolate of *Phytophthora capsici* Leonian used in this research, a bioassay was conducted under sterile conditions to look at infection and loss of pepper seedlings (*Capsicum annuum* L.) after inoculation and incubation. The isolate of *Phytophthora capsici* was originally identified growing on chili peppers in New Mexico.

Materials and Methods

Mature fruit of *Capsicum annuum* L. 'Thai Poinsettia', a locally grown ornamental pepper, were collected from the Texas A&M University Horticultural Gardens in September 2006 (College Station, Texas). Seed were extracted from the fruit, placed on paper towels and allowed to air dry for several days. On 15 November, 2006, seed were then surface sterilized by a 5 min. soak in a 2400 $mg \cdot L^{-1}$ sodium hypochlorite solution (The Clorox Co., Oakland, Calif.) and then triple rinsed in autoclaved distilled water under aseptic conditions. Seed were then transferred (one seed each) to 2.5 x 15.0 cm borosilicate culture tubes (VWR International, West Chester, Pa.) containing 10 cm^{-3} medium grade vermiculite (Sun Gro Horticulture, Pine Bluff, Ark.) previously moistened and autoclaved. Vermiculite was moistened with sterile distilled water via a wash bottle and the seed were covered with vermiculite twice the diameter of the seed. Culture tubes were capped and wrapped with paraffin film (Parafilm "M", American Can Co., Greenwich, Conn.) and placed in an incubator oven set at 25° C for 5 d to stimulate germination. After 5 d, tubes were transferred to slanting test culture tube racks on a laboratory bench under 35 $\mu mole \cdot m^{-2} \cdot s^{-1}$ PAR fluorescent light and ambient temperature (22 ± 2° C) and allowed to germinate. After the first true leaves formed (about 2 weeks), 15 tubes containing seedlings were inoculated with 1 ml of water containing mycelial fragments and zoospores of *Phytophthora capsici* at a concentration of $1 \times 10^5 \cdot ml^{-1}$. Another 15 tubes containing only seedlings without spore inoculum acted as a control (2 treatments x 15 replications per treatment = 30 tubes total).

Sporangium along with associated mycelia fragments were serially diluted by excising a 1 cm^{-2} piece of agar and transferring to a sterile 50 ml capacity conical bottom plastic tube with screw tight cap (BD Falcon, Fisher Scientific, Hampton, N.H.). Fifty ml of autoclaved distilled water was added to the tube and gently agitated to disperse the organisms. A 1 ml sample of this tube was then pipetted into another conical tube and 50 ml of sterile water was added and gently agitated. A pipette was used to place a sample on a hemacytometer with Neubauer markings and a maximum volume of 0.1µ L (Cole Parmer Instruments, Vernon Hills, Ill.) and placed under a light compound microscope (Binocular Compound Microscope, Carl Zeiss Inc., Thornwood, N.Y.) at 50 to 100 X magnification for quantification. The number of spores per ml of sample was calculated

by counting spores within the Neubauer markings multiplied by a dilution factor (50 ml) and multiplied by 10,000. Dilution continued until a spore count of about 1 x $10^5 \cdot ml^{-1}$ was obtained. After inoculation, tubes were capped and sealed with paraffin film and returned to the bench under light as before and observed for signs of disease which was defined as seedling stem collapse or lose of turgor indicating a loss of intact roots, and necrosis. At the end of 2 weeks, the number of surviving seedlings was recorded. The experiment was initiated on 1 December, 2006 and terminated on 15 December, 2006. The treatments were randomized within each test tube rack, and the test tube racks were randomly placed under the fluorescent lights on the bench top. Results were treated as categorical data and analyzed in SAS using PROC FEQ for tabular data using Chi-Square procedures to test for significance (SAS 9.1 for Windows, SAS Institute, Cary N.C.)

Results and Discussion

The results of this experiment at 14 d after inoculation with spores of *Phytophthora capsici* showed about a 75% incidence of disease resulting in pepper seedling death (Table 1). No significant ($P \leq 0.05$) incidence of seedling death occurred at 7 d, but death was significant ($P \leq 0.05$) at 10 and 14 d. The 10 to 14 d incubation period is a typical time interval for infection caused by *Phytophthora* to occur (Erwin and Ribeiro, 1996). Seedlings not inoculated with spores showed a 100% survival rate across all days after inoculation. Surviving seedlings in inoculated tubes may have lacked the necessary physical or environmental conditions for infection, or may be showing some resistance to the pathogen. With the exception of the seedlings intentionally inoculated with spores of *Phytophthora*, this experiment was conducted under sterile conditions in a laboratory environment instead of inoculating seedlings in an open or exposed greenhouse environment. The isolate of *Phytophthora capsici* used throughout the various studies maintained viability and virulence.

Table 1. Effect of inoculum and virulence of *Phytophthora capsici* on seedling survival of *Capsicum annuum* 'Thai Poinsettia' under sterile conditions at 1 to 14 d incubation.

		Days after inoculation			
Spore count [y]	Seedling	1	7	10	14
0	Live	15[z]	15	15	15
0	Dead	0	0	0	0
$1x10^5$	Live	15	13	7	4
$1x10^5$	Dead	0	2	8	11

[y] Spore estimate per ml water using hemacytometer determination.

[Z] Number of surviving or dead seedlings out of 15 possible; Values differed significantly ($P \leq 0.05$) from expected tabulated values at 10 and 14 d using Chi-square analysis, but were not significant at 1 or 7 d.

CHAPTER VIII
EFFECT OF OZONE TREATED WATER ON VEGETATIVE GROWTH OF CHRYSANTHEMUM

Atmospheric ozone gas is a common pollutant in industrialized urban centers or in areas where environmental conditions are favorable for ozone concentration buildup (Jacobsen and Hill, 1970). A concern in using ozone gas in water treatment is "off-gassing" or the process where excess ozone gas is vented to the atmosphere as a pollutant (Gottschalk et al., 2000). Elevated levels of ozone gas in the atmosphere can enter through leaf stomata and oxidize plant tissue to induce ozone damage. Symptoms vary, but may include foliar discoloration of leaves, chlorosis, and necrosis (Jacobsen and Hill, 1970). While this type of foliar damage can be caused by many factors other than ozone, additional types of damage have been linked to ozone. Flecks, stippling, bronzing, bleaching, or reddening of foliage has been reported (Jacobsen and Hill, 1970). Continual exposure or high concentrations of ozone can result in necrosis or chlorosis obscuring the above mentioned symptoms (Fiscus et al., 2005). Symptoms usually occur interveinally on the upper leaf surface but may affect both leaf surfaces (Fiscus et al., 2005). Older foliage is generally affected first with subsequent damage to younger tissue (Fiscus et al., 2005). Young plants are more sensitive to ozone while older mature plants are more resistant (Fiscus et al., 2005). Ozone exposure can also stunt plant growth and affect flower bud formation as well as causing marginal leaf rolling and scorching (Fiscus et al., 2005). Ozone exposure for 4 h at concentrations of 0.04 to 1.0 mg· L^{-1} induced injury symptoms (Sikora and Chappelka, 2004).

When considering ozone as a disinfecting agent in irrigation water, it is important to know if the possibility exists of inadvertently causing crop damage by ozone interacting with roots or shoots during irrigation with ozonated water or by possible off-gassing from excess ozone. On the other hand, a natural breakdown product of ozone is elemental oxygen which might have a positive affect on plant growth by increasing the oxygen content in the plant growing substrate. Chrysanthemum (*Chrysanthemum* x *morifolium* T. de Romatuelle) has been reported to be sensitive to ozone exposure (Sikora and Chappelka, 2004). Chrysanthemum is commonly grown in enclosed greenhouses

where the possibility exists of ozone concentration building up if off-gassing is occurring out of the irrigation water. The purpose of this experiment was to investigate potential positive, negative, or neutral affects of ozone treated irrigation water on garden chrysanthemum, at 3 nitrogen (N) levels using a complete water soluble fertilizer with micronutrients. Multiple N levels were used to test if ozone interacted at zero, low or high nutrient concentrations to affect vegetative plant growth. Additionally, chrysanthemum plants were directly exposed to ozone gas in a sealed container to document acute ozone damage.

Materials and Methods

Rooted cuttings (8 cm long) of *Chrysanthemum* x *morifolium* 'Country Girl' were potted on 9 August, 2006 in 0.73 L (TLC, Branford, Ontario, Canada) containers using a peat-based substrate (Metro Mix 700, Sun Gro, Bellevue, Wash.) and randomly placed on a greenhouse bench under ambient air of 23º C ± 5º C, and a natural photoperiod of 700 µmole· m^{-2}· s^{-1} PAR as measured at 1:00 p.m. in mid-August 2006. Aliquots (1 L) of reverse osmosis (RO) water were amended with 0, 50, and 300 mg·L^{-1} N from a 21N-7P-7K water soluble fertilizer (Peter's Acid Special. Scott's Co., Marysville, Ohio) and exposed to ozone (O_3) gas to obtain peak ozone concentrations of 0, 0.5, 1.0 or 1.5 mg·L^{-1}. Ozone concentration was determined by the indigo colorimetric method. This procedure is based on the rapid and stoichiometrical decolorization of indigo trisulfonate (from indigo blue to clear) when in the presence of ozone (Bader and Hoigne, 1986). The source of the indigo trisulfonate was AccuVac ozone reagent low (0-0.2 mg·L^{-1}) and high range (0.25-1.0 mg· L^{-1}) evacuated ampuls (Hach Co., Loveland, Colo.). The tip of an ampul containing a measured amount of reagent is inserted into a sample of the ozonated water and crushed. The vacuum within the ampul draws in a 15 ml amount of the ozonated liquid. The ampul is inverted twice to insure mixing and then immediately placed in a portable ozone meter (Hach Co., Loveland, Colo.) and the ozone concentration is read directly as mg·L^{-1} O_3. For concentrations greater than 1.0 mg·L^{-1} O_3, a color comparison wheel (Hach Co., Loveland, Colo.) was used to determine O_3 concentrations up to 1.5 mg·L^{-1}. Ozone gas source was from an experimental catalytic electrochemical stack generator with a rated output of 0.113 kg· d^{-1} (Lynntech Industries, College Station, Texas). Ozone generator operating parameters were set at 9 amp output

with an ozone flow rate of 5 $ml \cdot L^{-1} \cdot s^{-1}$. Time to reach peak O_3 concentrations of 0, 0.5, 1.0, or 1.5 $mg \cdot L^{-1}$ in the 1 L fertilizer solutions via mass transfer were determined by prior indigo colorimetry assays to be 0, 60, 120, or 180 s, respectively. Aliquots were sealed for 15 min to allow for O_3 diffusion. Treated water was used to irrigate plants as needed until floral initiation which was 1 September, 2006. The experiment was arranged as a completely randomized design with a complete factorial of ozone (4 O_3 concentrations) and fertilizer (3 N concentrations) with 5 replicates per treatment combination (4 x 3 x 5 = 60 plants total). Data collected included an initial and final growth index (plant height times two perpendicular widths and reported as cm^3) and final shoot (g) and root dry mass (g) (60° C for 7 d). Chlorophyll content (chlorophyll a + b) was determination by the acetone extraction method as described by Harborne (1998) and reported as $mg \cdot mm^{-2}$ leaf area. Growth parameter (final growth index, shoot and root dry weight, chlorophyll content) data were analyzed for significance with fertility level and ozone concentrations as main effects and for possible interactions between fertility level and ozone concentration using SAS PROC GLM and means were compared using the least squares means procedure (SAS 9.1 for Windows, Institute, Cary, N.C.).

A "gas chamber" was constructed using a 100 L (volume) plastic storage bin (0.45 m x 0.80 m x 0.33 m) (Rubbermaid Inc, Fairlawn, Ohio) with a tight fitting lid. A small hole was drilled into one upper side to allow a feed line (65 mm outer diameter) to emit ozone gas into the sealed chamber. A safety precaution was taken to eliminate the inhalation of ozone gas by using a face respirator with activated charcoal filter canisters (Premier 6000 Respirator, Willson Co. Santa Ana, Calif.). Previously grown plants of 'Country Girl' chrysanthemum in 0.65 L pots were placed in the chamber and exposed to peak concentrations of 0, 0.50, 1.0, or 1.5 $mg \cdot L^{-1}$ ozone gas. Each ozone concentration had 5 single plant replicates (5 plants per ozone concentration x 4 ozone concentrations = 20 plants total). Exposure time to achieve required ozone concentrations was calculated by determining ozone gas feed rate (F_{O3}) in air which under the ozone generator operating parameters was 0.065 $mg \cdot L^{-1} \cdot s^{-1}$ O_3. Exposure times were calculated to be 7.6, 15.4, and 23.1 s, respectively, for desired O_3 concentrations. After treatment, plants were allowed to incubate in the chamber for 15 min. when acute toxicity symptoms were observed on the foliage. Plants were removed from the chamber and randomly placed on

a nursery bench under 50 % light exclusion for subsequent development of ozone damage symptomology. After 2 h, a total foliage damage visual rating of 1 (81 to 100% of foliage exhibiting injury), 2 (61 to 80%), 3 (51 to 60%), 4 (21 to 50%), or 5 (0 to 20%) was assigned to each plant. Results were treated as categorical data and analyzed in SAS using PROC FEQ for tabular data using Chi-Square procedures to test for significance (SAS 9.1 for Windows, SAS Institute, Cary N.C.).

Results and Discussion

Chrysanthemum x *morifolium* 'Country Girl' plants exposed to acute doses of ozone gas from 0 to 1.5 $mg \cdot L^{-1}$ started exhibiting typical ozone damage symptoms within 15 min. of exposure. After a 2 h development period, any exposure greater than 0.5 $mg \cdot L^{-1}$ showed foliar damage (Table 2). Damage symptoms included bronzing of the older mature leaves with necrosis subsequently developing (Fig. 7). Acropetal growth near the meristem area did not exhibit damage symptoms at 2 h. Some marginal leaf rolling occurred as well as bleaching of the foliage. These symptoms are similar in description to other reports of ozone damage to plants (Jacobsen and Hill, 1970). Results of this experiment suggest that treating water containing fertilizer solutions with ozone does not affect vegetative plant growth of chrysanthemum. No foliar symptoms of ozone damage were observed on the plants. However, chrysanthemum plants with induced ozone damage by direct exposure to ozone gas did display typical ozone damage symptoms. Results of the experiment using ozonated irrigation water to test for phytotoxicity on chrysanthemum indicated no significant interactions ($P \leq 0.05$) between ozone concentration and fertility levels for shoot dry mass, root dry mass, total plant dry mass, root to shoot ratio, final growth index, or chlorophyll content. Significant differences ($P \leq 0.05$) did occur in shoot and root dry mass and chlorophyll content among fertility levels across ozone concentrations, but not for ozone concentrations across fertility levels (Table 3). Increasing fertility levels increased all growth parameters, including chlorophyll content. Increasing ozone concentration did not negatively impact any of the growth parameters, nor did it positively impact growth. Chlorophyll content was unaffected by increasing ozone concentration. Micronutrient or other mineral deficiencies, as well as non-specific root damage are often associated with chlorosis or leaf yellowing and is a commonly diagnostic feature when looking at plant

health. These results suggest that ozonated irrigation water used routinely for irrigation does not adversely affect vegetative growth of the ozone sensitive species *Chrysanthemum* x *morifolium* at concentrations up to 1.5 $mg \cdot L^{-1}$ O_3.

Figure 7. Foliar damage symptoms on *Chrysanthemum* x *morifolium* 'Country Girl' caused by ozone gas for 15 min. at a concentration of 1.5 mg· L^{-1}.

Table 2. Effects of direct ozone gas exposure on subsequent foliar damage on *Chrysanthemum* x *morifolium* 'Country Girl'. Plants exposed to ozone concentrations (mg· L^{-1}) in a sealed chamber for 15 min. Ratings were assessed 2 h after removal from chamber.

Ozone	Foliar Damage Rating[y]				
(mg·L^{-1})	1	2	3	4	5
0	0[z]	0	0	0	5
0.5	0	0	0	4	1
1.0	1	0	2	2	0
1.5	4	0	1	0	0

[y] Rating scale corresponding to a percent of foliage damaged: 1 (81 to 100%), 2 (61 to 80%), 3 (41 to 60%), 4 (21 to 40%), 5 (0 to 20%).

[z] Number of plants out of 5 possible; Two-way tabular analysis using Chi-squares was significant at $P \leq 0.05$.

Table 3. Main effects ($P \leq 0.05$) of ozone treated irrigation water on shoot dry mass, root dry mass, total plant dry mass, root to shoot ratios, final growth index, and chlorophyll on *Chrysanthemum* x *morifolium* 'Country Girl' with increasing fertility levels (N). Values are means of 20 observations.

	Dry mass					
N level ($mg \cdot L^{-1}$)	Shoot (g)	Root (g)	Total (g)	Root to Shoot Ratio ($g \cdot g^{-1}$)	Growth index (cm^3)	Chlorophyll ($mg \cdot m^2$)
0	1.01[z]a	1.03 a	2.04 a	1.01 a	522.55 a	0.047 a
50	1.29 b	1.43 b	2.72 b	1.10 b	654.35 b	0.056 b
300	2.17 c	2.49 c	4.66 c	1.14 c	2574.00 c	0.098 c

[z] Means followed by the same letter are not significantly different at $P \leq 0.05$ using least squares means procedures.

CHAPTER IX
OZONE INTERACTIONS WITH FERTILIZER SOLUTIONS

Because ozone is a strong oxidizer (causing a loss of electrons with a subsequent increase in oxidation number in the element being oxidized), ozone has the potential to react with metals contained in plant fertilizers (Runia, 1994; Vanachter et al., 1988). Of particular interest is iron (Fe) which is classified as a transition metal. Transition metals are elements whose atoms have incomplete d sub-shell electron orbitals, or which yield cations with incomplete d sub-shells (Brown and LeMay, 1977). Transition metals are important because they can have various oxidation states and can form complexes and act as catalysts (Brown and LeMay, 1977). These traits are important in biological reactions. Other transition metals found to be essential for plant growth include manganese (Mn), nickel (Ni), copper (Cu), and zinc (Zn) (Marschner, 1995). Elemental iron is an electropositive metal (electronegativity 1.8 in the 2+ valence state) and can therefore readily donate electrons to a highly electronegative element such as the oxygen liberated during the ozone decomposition process. The oxidation of iron is summarized by the reaction $Fe^{2+} \rightarrow Fe^{3+} + e^-$ (Brown and LeMay, 1977). The oxidized form of iron (Fe^{3+}) is not readily taken up by plant roots and must be chemically reduced by various mechanisms to be biologically active in plants (McDonald, 1990). Major nutritional elements such as nitrogen, phosphorus, and potassium have not been found to be negatively affected by ozone (Ehret et al., 2001; Runia, 1994). Other possible effects of interest include possible change in solution pH and electrical conductivity (EC). Because of the importance of iron as a micronutrient in plant growth, and the possibility of ozone interaction with fertilizer application by irrigation water, a series of studies were undertaken to ascertain possible affects of ozone on plant nutrient constituents contained in fertilizer solutions typically used for commercial plant production.

Materials and Methods

Ozonated aliquots of distilled water containing a common commercially used water soluble fertilizer were measured for nitrogen content in both the nitrate (NO_3^-) and ammonium (NH_4^+) forms in addition to phosphorus (P), potassium (K), ferrous iron (Fe^{2+}) and ferric iron (Fe^{3+}), and total iron content. Electroconductivity and pH were also measured.

Aliquots (50 ml) of distilled water were each amended with 0, 50, 100, or 300 mg· L^{-1} N from a water soluble acid forming fertilizer containing 21N-7P-7K (Peters Acid Special, Scott's Co., Marysville, Ohio). This fertilizer is formulated to have Fe-EDTA, a chelated form of Fe^{2+} containing 0.714 mg· L^{-1} at 100 mg· L^{-1} N solution. Aliquots were placed in 50 ml conical bottom plastic tubes with screw tight caps (BD Falcon, Fisher Scientific, Hampton, N.H.). Tubes containing fertilizer solutions were bubbled with ozone gas via a gas diffusion airstone (Top Fin Air Stone, Pacific Coast Distributing, Phoenix, Ariz.) to obtain peak concentrations of 0, 0.5, 1.0, or 1.5 mg·L^{-1} O_3. Total aliquots tested were 4 fertilizer concentrations x 4 ozone concentrations x 5 replications each for a total of 80 water samples per nutrient analysis. Ozone concentration was determined by the indigo colorimetric method whereby a indigo blue reagent is progressively oxidized to a clear solution in the presence of increasing ozone concentration. The methodology uses AccuVac ozone reagent low (0-0.2 mg·L^{-1}) and high range (0.25-1.0 mg· L^{-1}) (Hach Co., Loveland, Colo.) evacuated ampuls. The tip of an ampul containing a measured amount of reagent is inserted into a sample of the ozonated water and crushed. The vacuum within the ampul draws in a 15 ml amount of the ozonated liquid. The ampul is inverted twice to insure mixing and then immediately placed in a portable ozone meter (Hach Co., Loveland, Colo.) and the ozone concentration is read directly as mg·L^{-1} O_3. For concentrations greater than 1.0 mg·L^{-1} O_3, a color comparison wheel (Hach Co., Loveland, Colo.) was used to determine O_3 concentrations up to 1.5 mg·L^{-1}. Ozone generator operating parameters were set at 9 amp output with an ozone flow rate of 5 ml·L^{-1}· s^{-1}. Time to reach peak O_3 concentrations of 0, 0.5, 1.0, or 1.5 mg·L^{-1} in the fertilizer solutions via mass transfer were 0, 30, 60, or 120 s, respectively, at the set parameters. Immediately after treatment, tubes were sealed and

allowed to incubate for 1 h. Samples were placed in a light free environment at 5 ºC until individual nutrient analysis was performed.

For all subsequent nutrient analysis, the same general procedure was used. From each ozone/fertilizer treatment combination, a total of 80 samples were analyzed per nutrient of interest. Aliquots of 50 mls were sufficient in volume to carry out required analysis. Results are reported in mg· L^{-1} nutrient and means were analyzed using a general linear model (PROC GLM) to analyze main effects and interactions, and means comparisons were made using least squares means procedures in SAS (SAS 9.1 for Windows, Institute, Cary, N.C.).

Nitrogen Content: Nitrate (NO_3^-) content was determined by a colorimetric procedure using salicylic acid (Cataldo et al., 1975) which yields a yellow color upon reaction with nitrate. Standard curves were obtained using $NaNO_3$ as a nitrate source. Samples were read on a spectrophotometer (Spectronic 20, Fisher Scientific, Hampton, N.H.) at 410 nm and reported as mg· L^{-1} NO_3^-. Ammonium (NH_4^+) concentration was determined by using an ion-selective probe to directly read from treated samples (Ammonium ion-selective probe, Cole-Parmer Instrument Co., Vernon Hills, Ill.) with standard curves using NH_4SO_4 as an ammonium source and reported as mg· L^{-1} NH_4^+. The fertilizer used in this experiment was an acid forming formulation containing very low nitrate levels with ammonia being the main source of nitrogen. To test the effects of ozone on nitrate forms of nitrogen, a separate nitrate analysis was performed using potassium nitrate (KNO_3^-) as a nitrogen source using the analytical procedure as described above (Cataldo et al., 1975).

Phosphorus Content: Phosphorus (P) was measured by a colorimetric procedure by Strickland et al. (1968) which produces a blue color in the presence of phosphorus. Standard curves were generated using potassium phosphate (KH_2PO_4) as a standard and results reported as mg· L^{-1} P.

Potassium Content: Potassium (K) was measured by placing one drop of each sample on the sensor pad of an ion specific meter (Cardy K^+ ion meter, Horiba Americas, Irving, Calif.) which directly reads sample concentration as mg ·L^{-1} K.

Iron Content: Samples treated as above were placed in amber colored bottles (250 ml capacity) and kept on ice with light exclusion until transported to a local testing

laboratory (Thompson Analytical, College Station, Texas) for subsequent iron determinations. Ferrous (Fe^{2+}) and Ferric (Fe^{3+}) content was measured. The methodology used was the phenanthroline method (Clesceri et al., 2005). Results are reported as mg· L^{-1} Fe^{2+} , Fe^{3+}, and $Fe^{2+} + Fe^{3+}$.

Electrical Conductivity (EC): Total dissolved salt content was measured by the EC of the samples read directly by a portable EC meter (EC meter, Horiba Americas, Irving, Calif.). Results are reported as microsiemens ($\mu S \cdot cm^{-1} \cdot s^{-1}$).

Solution pH: The solution pH was directly read by using a portable pH meter (Horiba, portable pH meter, Horiba Americans, Irving, Calif.) to sample each treatment. Results are reported in pH units.

Results and Discussion

The results of the analysis of the fertilizer components nitrate (NO_3^-) and ammonical (NH_4^+) nitrogen, phosphorus (P), potassium (K), electrical conductivity (EC), and pH of the test fertilizer solution show no significant interactions ($P \leq 0.05$) between increased fertilizer and ozone concentrations in a complete fertilizer with N primarily in the ammonical form (Table 4). There was a significant difference ($P \leq 0.05$) in the main effect of fertilizer concentration with the NH_4^+, P, K, and EC fertilizer parameters, but not in increasing ozone concentrations. There was also a significant difference ($P \leq 0.5$) in the lowering of solution pH at increasing fertilizer concentrations, but not increasing ozone concentrations. This pH lowering is expected in an acid forming fertilizer. Results also did not show a significant ($P \leq 0.05$) interaction between ozone levels and increasing nitrate content on an additional analysis using a KNO_3^- fertilizer source. There was a significant difference ($P \leq 0.05$) in NO_3^- concentration across increasing fertility levels, but not in increasing ozone concentration (Table 4). Iron in the reduced ferrous (Fe^{2+}) form did not show a significant interaction ($P \leq 0.05$) with increasing ozone concentration. Detectable Fe^{2+} levels overall were low (0.03 mg· L^{-1} maximum detected) and just above the assay detectability limit of 0.02 mg· L^{-1} (data not shown). Either the iron in the fertilizer was already in an oxidized ferric (Fe^{3+}) state or the reduced Fe^{2+} chelated form of iron in Fe-EDTA is not detectable using the selected analytical procedure. At 1.5 mg· L^{-1} ozone, detectable Fe^{2+} levels did decrease to 0 mg·L^{-1} across all fertilizer concentrations. However, Fe^{3+} content and increasing ozone concentration

was significant at 50 and 100 mg· L^{-1} N levels with a slight increase in Fe^{3+} content at 1.5 mg· L^{-1} ozone over the 0.0 mg·L^{-1} ozone concentration (Fig. 8). The iron in the test fertilizer is formulated to contain 0.35 mg· L^{-1} and 0.714 mg· L^{-1} Fe-EDTA at 50 and 100 mg· L^{-1} N respectively. The detectable amounts of iron were slightly above the formulated amounts of iron indicating possible variation in fertilizer manufacturing or blending. At 300 mg· L^{-1} N, the initial Fe^{3+} iron content of 12.79 mg·L^{-1} is about 3 times the 100 mg· L^{-1} N level of 4.21 mg· Fe^{3+} as was expected. At 0.5 and 1.0 mg· L^{-1} ozone at the 300 mg· L^{-1} N rate, Fe^{3+} content drops to 8.25 and 7.87 mg· L^{-1}, respectively. At 1.5 mg· L^{-1} ozone at the 300 mg· L^{-1} N rate, Fe^{3+} content decreased to 0 mg· L^{-1}. Total iron content followed a similar trend as Fe^{3+} since this form of Fe was the main component of the fertilizer test solution (Fig. 9). At 300 mg· L^{-1} N and the corresponding higher initial total iron content of 12.80 mg· L^{-1}, increasing ozone concentration decreased iron content to 0 mg· L^{-1} at 1.5 mg·L^{-1} ozone (Fig. 9). There is a conundrum in the results with low to medium initial iron content as compared to the high initial iron content. Whereas the low to medium total iron content did not decrease to 0 mg· L^{-1} at the high ozone concentration, the high initial iron content did decrease with increasing ozone concentration to 0 mg·L^{-1}. One possible explanation is that Fe is often used as a catalyst in ozone reactions in industrial applications (Rice at al., 1986; Gottschalk, et al., 2000). The higher Fe content could possibly be auto-catalyzing the reaction and increasing the rate at which Fe itself is precipitated out of solution. Ozone is commonly used to precipitate high Fe concentrations from drinking water and industrial wastewaters (Rice et al., 1986). Results indicate that increasing ozone concentration does have an effect on the iron component of the test fertilizer, but does not affect the macro nutrients as N, P, K. Electroconductivity and solution pH were also unaffected ($P \leq 0.05$) by ozone concentrations used in this study (Table 4).

Table 4. Fertilizer parameter (NH_4^+, NO_3^-, P, K), fertilizer concentration ($mg \cdot L^{-1}$), electrical conductivity ($\mu S \cdot cm^{-1} \cdot s^{-1}$), and pH analysis of the effect of increasing ozone (O_3) and fertilizer concentration on a water soluble fertilizer.

Fertilizer Parameter	Fertilizer concentration ($mg \cdot L^{-1}$)	Ozone concentration 0.0 ($mg \cdot L^{-1}$)	0.5 ($mg \cdot L^{-1}$)	1.0 ($mg \cdot L^{-1}$)	1.5 ($mg \cdot L^{-1}$)
NH_4^+	0	0.0	0.0	0.0	0.0
	50	84.5	81.8	85.0	83.6
	100	186.0	187.0	187.6	188.0
	300	320.3	312.0	320.6	315.3
Anova effects					
NH_4^+	*[z]				
O_3	n.s.				
NH_4^+ x O_3	n.s.				
NO_3^-	0	0.6	0.7	0.5	0.7
	50	48.3	52.9	54.4	54.1
	100	101.8	98.4	98.8	95.5
	300	287.8	286.3	284.7	285.5
Anova effects					
NO_3^-	*				
O_3	n.s.				
NO_3^- x O_3	n.s.				
P	0	0.4	0.9	5.0	2.4
	50	19.1	19.3	18.6	16.4
	100	33.6	32.6	33.0	44.4
	300	87.5	90.5	95.2	94.0
Anova effects					
P	*				
O_3	n.s.				
P x O_3	n.s.				
K	0	0.0	0.0	0.0	0.0
	50	12.3	12.0	12.0	11.0
	100	29.0	29.0	28.3	28.4
	300	82.0	82.0	80.6	82.3
Anova effects					
K	*				
O_3	n.s.				
K x O_3	n.s.				

Table 4. Continued.

Fertilizer Parameter	Fertilizer concentration ($mg \cdot L^{-1}$)	Ozone concentration 0.0 ($mg \cdot L^{-1}$)	0.5 ($mg \cdot L^{-1}$)	1.0 ($mg \cdot L^{-1}$)	1.5 ($mg \cdot L^{-1}$)
Electrical conductivity	0	0.004	0.004	0.005	0.003
	50	0.290	0.300	0.310	0.266
	100	0.620	0.636	0.633	0.630
	300	1.570	1.600	1.600	1.590
Significance					
Fertilizer	*				
Ozone	n.s.				
Fertilizer x Ozone	n.s.				
pH	0	6.53	6.23	5.90	5.92
	50	6.00	5.66	5.70	5.90
	100	5.60	5.56	5.40	5.63
	300	5.43	5.35	5.33	5.40
Significance					
Fertilizer	*				
Ozone	n.s.				
Fertilizer x Ozone	n.s.				

[z] Significant at $P \leq 0.05$, or n.s.= not significant.

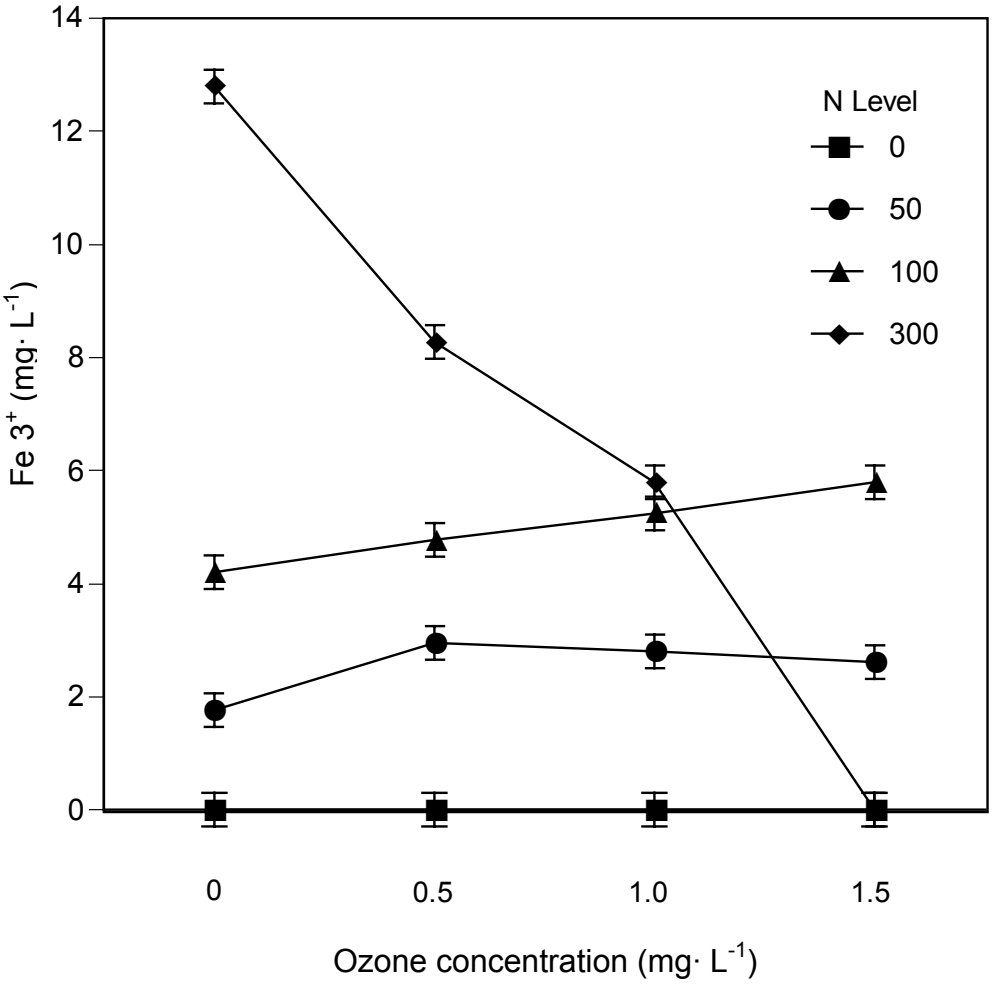

Figure 8. Effect of increasing fertility level (expressed as the N level in $mg \cdot L^{-1}$) and ozone concentration on ferric (Fe^{+3} in $mg \cdot L^{-1}$) content. Symbols represent means ± standard errors of 5 observations.

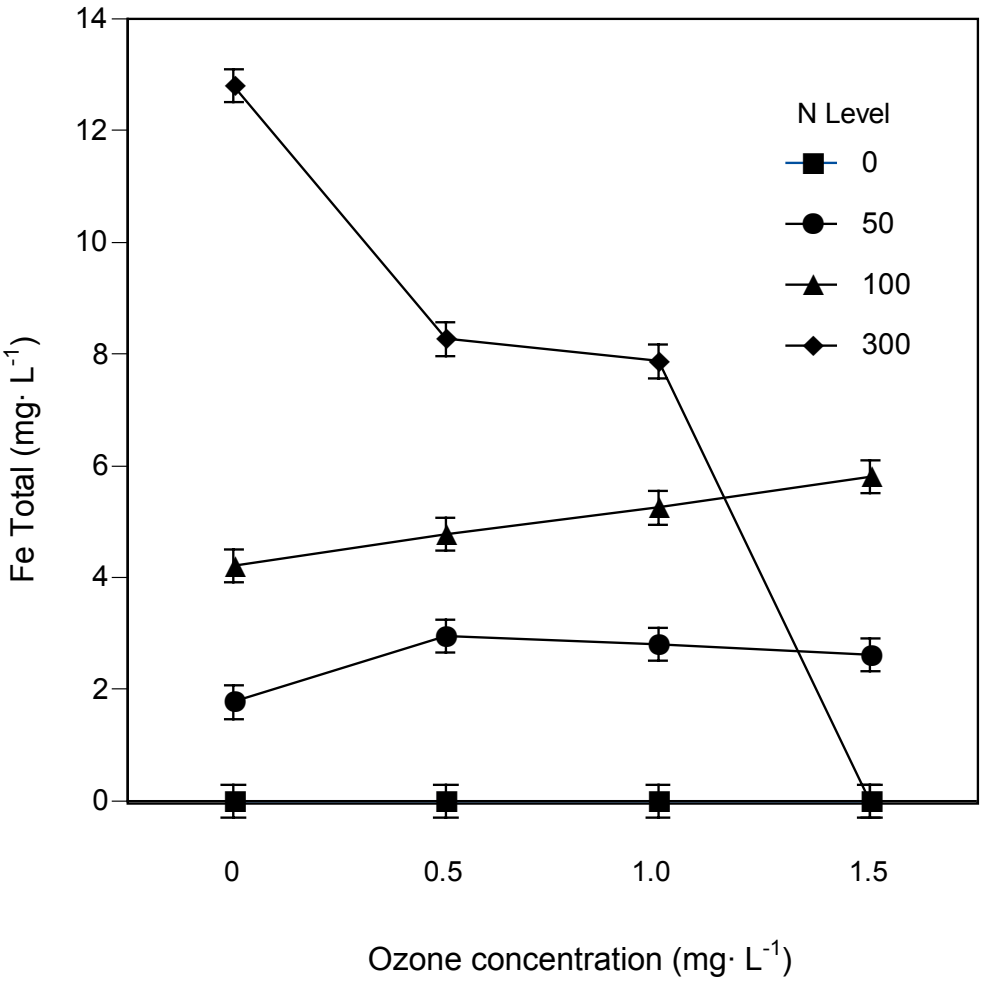

Figure 9. Effect of increasing fertility level (expressed as the N level in mg· L^{-1}) and ozone concentration on total Fe content (Fe total in mg· L^{-1}). Symbols represent means ± standard errors of 5 observations.

CHAPTER X
PRELIMINARY STUDY ON OZONATION OF A PLANT GROWTH REGULATOR ON PANSY

Chemical plant growth regulators (PGR) are an important component of greenhouse and nursery production systems. They are used to reduce plant height or otherwise retard growth making plants more compact, aesthetically pleasing, easier to ship, or prolong marketable shelf life. Most act by inhibiting gibberellic acid synthesis or otherwise retard stem elongation and growth (Rademacher, 2000). Many of these chemicals are highly active stable compounds with long residual times in plant tissue (Arnold and McDonald, 2005). These chemicals are also active at low concentrations (Douglas and Paleg, 1974). Post-production carryover or lag time effects have been documented in a number of ornamental species long after they have been planted to the landscape (McDonald and Arnold, 2005). At higher concentrations or with multiple applications during the production phase, landscape performance can be negatively affected (Arnold and McDonald, 2001; McDonald and Arnold, 2005). Plant growth regulators have also been found in recycled nutrient solutions from ebb and flow irrigation of pot plants (Adriansen, 1997). Because of the wide-spread use and the effectiveness of PGR's in plant tissue at low concentrations, the potential exists that recycled irrigation water can become contaminated with PGR's and redistributed throughout the production facility, or discharged with the potential to become a non-point source pollutant. Ozone gas has been used under high concentrations and under catalytic conditions to chemically oxidize organic waste, and deactivate certain pesticides and herbicides (Rice, 1981; Rivas et al., 2001; Runia, 1994). The purpose of this experiment was to study the possibility of ozone gas (at levels used to control pathogens) deactivating a commonly used PGR, paclobutralzol, in greenhouse bedding plant production.

Materials and Methods

Nontreated pansy (*Viola* x *wittrockiana* H. Gams 'Bingo Blue with Blotch') plugs were obtained from the Texas Agricultural Research and Extension Center, Overton, Texas on 15 October, 2006 and transplanted into 0.57 L containers (TLC, Branford, Ontario, Canada) using a peat based substrate (Metro-Mix 700, Sun-Gro Horticulture, Bellevue, Wash.) and placed on a greenhouse bench with an average mid-day light level of 350 μmole · m^{-2} · s^{-1} PAR and setpoints at 25° C/21° C day/night temperatures. Plants were watered as needed with reverse osmosis (RO) water and fertilized with a water soluble 300 mg· L^{-1} fertilizer (21N-7P-7K Peter's Acid Special, Scott's Co., Marysville, Ohio) twice weekly. At day 14, plants were either sprayed with distilled water to runoff (10 ml per plant) or sprayed (10 ml per plant) with paclobutrazol [(2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethly-2-1, 2, 4-triazol-1-yl-pentan-ol, formulated as Bonzi, Uniroyal Chemical Co. Middlebury, Conn.] at 15 mg· L^{-1} that had been treated with ozone at 0.0, 0.5, 1.0, or 1.5 mg· L^{-1} peak O_3 concentration after paclobutralzol addition. Ozone concentration was determined by the indigo colorimetric method. This procedure is based on the rapid and stoichiometrical decolorization of indigo trisulfonate (from indigo blue to clear) when in the presence of ozone (Bader and Hoigne, 1986). The source of the indigo trisulfonate was AccuVac ozone reagent low (0-0.2 mg·L^{-1}) and high range (0.25-1.0 mg· L^{-1}) evacuated ampuls (Hach Co., Loveland, Colo.). The tip of an ampul containing a measured amount of reagent is inserted into a sample of the ozonated water and crushed. The vacuum within the ampul draws in a 15 ml amount of the ozonated liquid. The ampul is inverted twice to insure mixing and then immediately placed in a portable ozone meter (Hach Co., Loveland, Colo.) and the ozone concentration is read directly as mg·L^{-1} O_3. For concentrations greater than 1.0 mg·L^{-1} O_3, a color comparison wheel (Hach Company, Loveland, Colo.) was used to determine O_3 concentrations up to 1.5 mg·L^{-1}. Ozone gas source was from an experimental catalytic electrochemical stack generator with a rated output of 0.113 kg· d^{-1} (Lynntech Industries. College Station, Texas). Ozone generator operating parameters were set at 9 amp output with an ozone flow rate of 5 ml·L^{-1}· s^{-1}. Five replicates per treatment combination (2 PGR concentrations x 4 O_3 concentrations x 5 replications per treatment combination = 40 plants total) were completely randomized on a greenhouse bench. Heights and canopy

diameter in two perpendicular directions were initially measured at treatment time. A plant index was calculated as height x width 1 x width 2 as a volume estimate of canopy size and reported as growth index (cm^3). Five plants were also initially harvested for a baseline dry matter determination. At first flower, 20 November 2006, the study was terminated. Final growth measurements were taken and the plants were harvested for shoot and root dry mass (60° C for 7 d). Means of plant index, shoot and root dry mass were analyzed using a general linear model (PROC GLM) to analyze main effects and interactions, and means comparisons were made using least squares means procedures in SAS (SAS 9.1 for Windows, Institute, Cary, N.C.).

Results and Discussion

No significant ($P \leq 0.05$) interactions were found for increasing ozone concentrations and PGR applications. Likewise, the main effect of ozone concentration was not significant ($P \leq 0.05$). However, the main effect of paclobutralzol application (Table 5) was significant ($P \leq 0.05$). Increasing ozone concentration from 0 to 1.5 $mg \cdot L^{-1}$ did not effect the efficacy of paclobutrazol in controlling growth of pansy when applied at 0 or 15 $mg \cdot L^{-1}$. These results suggest that ozone at the tested concentrations would not affect paclobutrazol applications administered to a target crop via irrigation systems. However, ozonation at the tested levels did not inactivate the paclobutrazol and prevent it from being potentially recycled or discharged. Ozone is commonly used to treat wastewater to remove organic and other compounds (Rice at al., 1986). In waste water treatment facilities, ozone dosages of up to 50 $mg \cdot L^{-1}$ are typical. The average dose for drinking water and the beverage industry is 0.5 to 1.2 $mg \cdot L^{-1}$ (Gottschalk et al. 2000). Further research is needed in investigating higher ozone concentrations and possibly a longer exposure times to test inactivation of paclobutrazol and other commonly used plant growth regulators.

Table 5. Main effects of paclobutralzol applications on growth of *Viola* x *wittrockiana* 'Bingo Blue with Blotch' grown for 36 days in 0.57 L containers in a greenhouse. Values represent means of 20 observations.

Paclobutrazol ($mg \cdot L^{-1}$)	Final growth index (cm^3)	Shoot dry mass (g)
0	553.40[z] a	0.66 a
15	150.83 b	0.37 b

[z] Means followed by different letters are significantly different at $P \leq 0.05$ using least squares means procedures.

CHAPTER XI
SUMMARY

Results

The production of high quality horticultural products demands high quality water in copious quantities. Increased urbanization in many horticultural production areas coupled with increasing water demand and decreasing quality and quantity dictates that the horticulture industry conserve current and future water resources. Additional concerns such as overall water discharge, agricultural chemical contamination, and nutrient leaching into the groundwater or downstream surface water must be addressed. One strategy to mitigate runoff and conservation concerns is to capture and reuse existing irrigation water. A major concern in the reuse of irrigation water is the possibility of spreading plant pathogens via recycled irrigation water (Hong and Moorman, 2005; Thompson and Allen, 1974). Nursery production facilities and environments are normally favorable for disease development and proliferation. High humidity levels, standing water, and lack of air-circulation in growing areas are particularly conducive to the development of disease outbreaks caused by species of *Phytophthora*. Virulent species such as *Phytophthora ramorum* are especially problematic with a potential for widespread disease outbreaks and can impact not only production facilities, but landscapes and the general environment with significant economic and ecological consequences (Rizzo et al., 2002; U.S. Department of Agriculture, 2005). Traditional water treatment systems such as settling ponds and reservoirs, filtration, and even constructed wetlands can mitigate particulate matter or sediment accumulation and excess fertilizer discharge (MacDonald et al., 1994), but can't fully address the problem of pathogen dissemination. This problem of pathogen control has traditionally been addressed through the use of biocides or other disinfecting agents such as chlorination. Chlorination is an effective means of control as it has a long residual in solution (Ferraro and Brenner, 1997). However, many plants are sensitive to chlorine and production equipment may become corroded or degraded over time (Grasso, 1996). Chlorine is dangerous and may present health hazards if not handled properly (Grasso, 1996). Alternative methods to disinfect water supplies have been tried such as lasers, ultraviolet

light, peroxides, and filtration (Grasso, 1996; Ramsay, 2000). While these methods may be effective in certain applications, they have limitations in effectiveness and practicality in horticultural operations.

Ozone gas has been used to treat municipal water supplies in Europe since the late 1800's (Brink et al., 1991; Rideal, 1920). It is commonly used in treating industrial wastewater, bottled water supplies, and increasing being used in drinking water treatment plants (Rice et al, 1986). Ozone is a strong oxidizer and is used to control a wide range of microorganisms including viruses, bacteria, protozoa, and fungi in drinking water. Ozone is effective in low concentrations, usually < 1.0 mg· L^{-1} O_3 (Rice at al., 1986). Ozone generation has advanced with increased production efficiency using new electrochemical fuel cell technology (Flusche, 2006).

The purpose of this research project was to investigate the possible use of ozone gas to control a plant pathogen of interest to the horticultural industry. Species of *Phytophthora* deBary are especially problematic in the horticultural industry and were chosen as a test organism. *Phytophthora capsici* Leonian was chosen as a model pathogen as it infects a large number of cultivated plant species, both ornamental and olericultural (Erwin and Ribeiro, 1996). There were a number of questions to address. The first question to answer was the efficacy of ozone in reducing the number of propagules of *Phytophthora capsici* in irrigation water. The second question was the effect of turbid or cloudy water on ozone efficacy. The third area of concern was the ability of *Phytophthora capsici*, under extended *in vitro* culture, to maintain pathogenicity or the ability to infect plants. The fourth question was the possibility of induced ozone damage or toxicity on vegetative plant growth due to residual ozone off-gassing from treated water when used to irrigate plants. Acute toxicity from a direct exposure to ozone gas on a test plant species needed to be documented to verify symptomology of ozone damage. And finally, the fifth question to ask is given that ozone is an powerful oxidizer, what are the effects of ozonating fertilizer-containing or plant growth regulator solutions on various nutrient or plant growth regulator components of a commercial fertilizer or plant growth control program typically used in production facilities?

Results from the ozonation of solutions containing propagules of *Phytophthora capsici* indicate that increasing ozone concentrations decreased the number of surviving propagules, reducing the subsequent number of colony forming units that formed in culture. Ozone concentrations of 1.5 mg· L^{-1} prevented the growth of any new colony forming units in culture (Figs. 3 and 4). Multiple treatment dates verified the trend in propagule reduction as ozone concentration increased. These results indicate that ozonation is efficacious in reducing propagules of *Phytophthora capsici* in irrigation water.

The effect of turbid or opaque irrigation water on the efficacy of ozone on reduction of propagules of *Phytophthora capsici* was tested using bentonite clay as a model system. Bentonite clay has been used in prior research to look at turbidity effects on screening viruses and bacteria during the ozonation process (Boyce et al., 1981). In this study, bentonite clay was used at concentrations corresponding to Nephelometric Turbidity Units of 0 to 2 NTU. A 2 NTU level is above the Environmental Protection Agency's permitted levels in surface water sources. Results indicate that increasing bentonite levels to 2 NTU did not effect the ability of increasing ozone concentrations to reduce the number of propagules of *Phytophthora capsici* and subsequent regrowth *in vitro* (Figs. 5 and 6). Other research indicates the source of turbidity is more important than the level of turbidity (Hoff, 1978). Organic matter is found to absorb microorganisms thus providing a screen or shield to the applied ozone. Organic matter can also scavenge or use ozone (ozone demand) reducing the amount available for microorganism oxidation. Bentonite clay was not found to negatively affect the ozonation process (Figs. 5 and 6). Whereas organic matter may be filtered, screened, or otherwise trapped and removed from recycled irrigation water, fine particulate clay particles can pass through most filtration systems. These results indicate that clay particles such as bentonite clay do not adversely affect the ozonation process.

The isolate of *Phytophthora capsici* was originally obtained as a pure culture from the American Type Culture Collection. It was sub-cultured *in vitro* on a vegetable broth based artificial agar media for several months during the course of the various experiments. To test for the continued virulence of the isolate of *Phytophthora capsici* with assumed pathogenicity, a bioassay was conducted using ornamental pepper

Capsicum annuum L., the same species from which the organism was originally isolated. Ornamental peppers were grown under aseptic conditions from seed until the first true leaves emerged. Plants were then inoculated with propagules of *Phytophthora capsici* and incubated for 14 days. At the end of the test period, about 75% of the inoculated seedlings were dead while all non-inoculated controls remained alive (Table 1). Results indicate that the pathogen was still virulent and capable of infecting and killing ornamental pepper seedlings.

A concern in using ozone gas, especially in enclosed structures is the possibility of off-gassing or the evolution of ozone gas out of irrigation water when under high concentrations. This evolving gas may have phytotoxicity implications if the concentration is high enough to cause either root or foliar damage to plants. Conversely, the breakdown product of ozone is elemental oxygen which could cause increased root zone oxygen content and improved plant growth. To test for phytotoxicity or other effects, *Chrysanthemum* x *morifolium* T. de Romatuelle was grown in a greenhouse and watered with plus or minus ozonated water at a range of fertility levels and allowed to grow until floral initiation. Plant growth measurements were taken, along with leaf chlorophyll content. Results indicate that increasing ozone concentrations to 1.5 mg· L^{-1} did not affect plant growth negatively or positively, but were neutral (Table 2). Chrysanthemum plants directly exposed to ozone gas exhibited foliar damage (Fig. 7) similar to that reported in other species (Jacobsen and Hill, 1970). No foliage damaged corresponding to the induced damaged was observed on the plants irrigated with the ozonated water. Results indicate that ozonated irrigation water at concentrations up to 1.5 mg· L^{-1} O_3 did not have a negative effect on vegetative growth in chrysanthemum under normal production conditions.

Ozone is a strong oxidizer and is often used to remove heavy metals from industrial waste water. Another use of ozone is to remove iron from municipal or other drinking water systems to reduce iron staining in plumbing and household fixtures. The effect of ozonation on fertility of irrigation water using a commercial fertilizer in horticultural production was studied. The major nutrient components of the fertilizer solution were analyzed as well as the micronutrient iron. Results indicate that increasing ozone concentration to 1.5 mg· L^{-1} did not effect the macro nutrient components nitrogen,

phosphorus, or potassium. Ozonation also had no effect on electroconductivity or solution pH. Increasing ozone concentrations did have an effect on ferric and total iron content at a high concentration of fertilizer (Figs. 8 and 9). When complete fertilizer applied at 300 mg·L^{-1} nitrogen was exposed to 1.5 mg·L^{-1} O_3, iron content decreased to 0 mg· L^{-1} Fe and precipitated out of solution. It is suggested, since iron is apparently affected by increasing ozone concentrations, irrigation water be treated and ozone allowed to decay prior to the fertilizer injection stage.

Plant growth regulators are commonly used during horticultural production to control plant growth for various reasons. A consequence of this practice is the possibility of irrigation water becoming contaminated with these plant growth regulator chemicals. These chemicals are often active in low concentrations and are stable compounds with an extended period of activity in the treated plant (Arnold and McDonald, 2001). Contaminated water could be recycled and applied to plants sensitive to plant growth regulators or otherwise applied where not intended. In addition, contaminated water could be discharged off-site and have unintended consequences downstream. A solution of paclobutrazol, a commonly used plant growth regulator, at a rate of 15 mg· L^{-1} was ozonated at 1.50 mg· L^{-1} O_3 and applied to pansy (*Viola* x *wittrockiana* H. Gams). Untreated water was used as a control. Results indicate that ozone at 1.50 mg· L^{-1} did not have an effect on paclobutralzol (Table 5). Applications of paclobutrazol through irrigation systems would be unaffected. However, ozone at the tested concentration also would not inactivate or neutralize the paclobutrazol in any recycled irrigation water.

Discussion

Ozone can be an effective sterilent for *Phytophthora capsici* and perhaps other species of *Phytophthora* in nursery/greenhouse irrigation water with minimal potential for plant damage. As mentioned, ozone is used to control many species of coliform bacteria and other forms of microorganisms in municipal water supplies. Other plant pathogens found in irrigation waters that might be controlled by ozonation include, *Phythium, Rhizoctonia,* and *Fusarium*. Many other fungal organisms are pathogenic to plants, but most have not been reported as being identified in irrigation water so the value of ozonating water to control these organisms, many of them soil-borne, is questionable. Ozone may be effective in controlling bacterial plant pathogens species such as *Erwinia*

and *Pseudomonas*. However, little data exist on effective ozone concentrations and methodology for these pathogens and experimental data would be necessary in designing ozonation systems to insure pathogen control.

This reseach showed that bentonite clay did not affect the effacy of ozone on the control of *Phytophthora*. However, it should be mentioned that bentonite clay does not require an ozone demand. Irrigation water found in horticultural production facilities such as a greenhouse or nursery operation would most likely have some amount of solid or dissolved organic matter in the recirculated water despite treatment by filtration or settling ponds. Any organic matter, or inorganic chemical compounds, would have an ozone demand above that which is required for pathogen control and would require a much higher ozone concentration that the maximum 1.5 mg· L^{-1} O_3 used in these studies. Higher ozone concentrations require the use of specialized detection, monitoring, and injection equipment designed to deliver the required higher ozone concentrations. Corrosion resistant piping at the ozone injection site would also be needed. In addition, gas handling and off-gassing concerns increase and provisions must be made to neutralize excess ozone gas (heat or catalyst degradation) to comply with possible local and state air quality standards.

Chemical precipitaton of iron by ozonation suggests that injection of fertilizer solutions containing iron or other micronutrients should occur after ozone decay. Iron was chosen as a model micronutrient in these studies because of its ability to readily change oxidation states and because iron deficiency symptoms on plants are usually apparent at an early stage of a lack of plant available iron. Other essential plant micronutrients such as copper (Cu), manganese (Mn), nickel (Ni), and zinc (Zn) are chemically related to iron and may also be oxidized and precipitated out of an ozonated fertility solution. Many complete water soluble fertilizers contain a mixture of micronutrients or are applied as a separate micronutrient fertilizer. As with iron, allowing sufficient time for ozone decay would mitigate the oxidizing effects of ozone on these other plant micronutrients.

The increased use of chemical plant growth regulators to manipulate or control plant growth may have a real impact on recirculated irrigation water in the future. As mentioned, these chemicals are stable and active in low concentrations. Re-applying

water containing these chemicals may have many unintended consequences on both on-site production and water released downstream. Ozone has been used to inactivate many organic and inorganic chemicals. Ozone levels used in these studies did not inactivate paclobutralzol and experimental data are needed to determine effective ozone concentrations for commonly used plant growth regulators. It is difficult to make ozonation recommendations and system requirements for these types of chemicals without a known range of effective concentrations. More studies are needed looking at chemical degradation by ozonation, especially in the area of plant growth regulators and horticultural pesticides.

The economics of upscaling ozone treatment to full scale horticulture production facilities are problematic. There are no "one size fits all" solutions. Ozonation should be considered as part of an overall strategy to reduce plant pathogens in recirculated irrigation water with the concomitant risk of increased disease incidence. Because of the expense involved in installing and maintaining ozone equipment, irrigation water should be tested and monitored for pathogens to access the potential or desirability of ozone treatment. The quality of the recirculated irrigation water must be assessed as to turbidity levels, composition, as well as the presence of organic or inorganic constituents which will influence ozone demand. The volume of water to be treated must also be considered. Large volumes of water will considerably increase the ozone generation requirements and will determine the type and capacity of the generation equipment needed. Possibilites exist whereby selected use of smaller quantities of ozonated water can be used in critical aspects of production such as in plant propagation facilities, liner production areas, or in the production of high value or sensitive crops. Ozone is another tool that may be considered in the overall strategy of crop protection and irrigation water remediation.

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VITA

Garry Vernon McDonald received his Bachelor of Science degree in floriculture from Texas A&M University in 1983. In 1984, he spent 14 months studying tropical horticulture at Fairchild Tropical Garden in Miami, Florida. After 3 years in the landscape design and installation business, in 1998 he entered into a graduate program in floriculture at Texas A&M University. His research was in the area of plant nutrition studying the mechanism of iron uptake in rose rootstocks. He graduated with a Master of Science degree in 1990. Upon graduation he went to work as a Research Technician at the Texas Agricultural Research and Extension Center in Overton, Texas. His research at Overton was in the area of field and greenhouse rose production, bedding plant production, and plant introduction. In 1998, he transferred to the Texas A&M University main campus as a Research Associate and began his doctoral program where he received his PhD in horticulture in 2007. While pursuing his doctoral, he also was a teaching assistant in landscape construction and maintenance in addition to two different landscape plant material classes.

Dr. McDonald may be reached at the Department of Horticultural Sciences, Horticulture Forest Science Building, Texas A&M University, College Station, Texas 77843-2133. His email address is g-mcdonald@tamu.edu.