THESIS

INCREASED CHLOROPHYLL EFFICIENCY OF DARK-ADAPTED CAMELLIA FOLIAGE WHEN TREATED WITH CHLORINE DIOXIDE OR HYDROGEN DIOXIDE AND BLENDED WITH A NON-IONIC SURFACTANT

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ABSTRACT

INCREASED CHLOROPHYLL EFFICIENCY OF DARK-ADAPTED CAMELLIA FOLIAGE WHEN TREATED WITH CHLORINE DIOXIDE OR HYDROGEN DIOXIDE AND BLENDED WITH A NON-IONIC SURFACTANT

Phytophthora ramorum is a major risk to interstate trade of nursery stock. This work focused on chemical oxidant chemistry as a disinfectant of nursery grown camellia plants. Disinfection of nursery stock is crucial for shipping, but the impact on plant health and phytotoxic responses are also important. To determine plant stress responses to applied chemical oxidants, we measured chlorophyll activity (PSII maximum quantum efficiency) as measured by Fv/Fm values on dark-adapted camellia plants. Data were collected using a Li-COR 6400XT leaf chamber fluorometer (Li-COR, Lincoln, NE) to evaluate the potential phytotoxicity of camellia to foliar applied chlorine dioxide (ClO_2) and hydrogen dioxide (H_2O_2), with or without sarcosinate surfactant with consecutive spray applications. Chlorophyll activity (Fv/Fm) of dark adapted foliage was greater when ClO_2 and H_2O_2 were applied with sarcosinate surfactant to camellia foliage compared to treatments not containing sarcosinate surfactant. Chlorophyll activity decreased with increasing concentrations of ClO_2 without sarcosinate. Higher Fv/Fm across seven measurement intervals were observed in ClO_2 treatments compared to H_2O_2 treatments at the same concentration. Visual injury of camellia foliage increased with each of the five subsequent spray applications; however, foliar injury did not exceed a marketable threshold for most treatments, until after four consecutive spray applications at

400 mg·L^{-1} ClO_2, with or without surfactant. This study demonstrated that Electro-BioCide at a rate predicted to eradicate *Phytophthora ramorum* (200 mg·L^{-1}) should not visually damage camellia plants until after five consecutive spray applications. These findings indicate that Electro-BioCide has the potential to be implemented as a preventative foliar treatment for defense against foliar plant pathogens, without concern for detriment to plant health.

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INTRODUCTION

Native plant pathogens are a costly problem in ornamental plant industries around the world. However, exotic pathogens are especially damaging and can be rapidly moved great distances to many locations if nursery stock is infected (Frankel, 2008; Tjsvold et al., 2008; Tubajika et al., 2006). The United States nursery industry in particular has become exceptionally concerned with a variety of pathogens due to the widespread shipping of potentially infected plant material to regions ideal for growth and spread of disease.

With a continuous surge in the amount of plant material being shipped with increasing distances around the country and around the world, disinfection of plant material has become a top priority. Difficult pathogens to eradicate, including *Phytophthora ramorum*, make this challenge even more problematic. With a host range of over 135 species, identifying, containing, treating, and eradicating *P. ramorum* has proven to be a difficult task since its discovery in the United States in the 1990s (Frankel, 2008). To prevent further quarantines of nurseries around California, research has shifted towards finding a valid treatment method for nursery stock contaminated with *P. ramorum*.

Electro-BioCide (E-B) (Strategic Resource Optimization Inc. (SRO), Denver, CO) a patent-protected, EPA registered (87492-1), general use oxidant disinfectant, composed of a proprietary blend of chlorine dioxide (ClO_2), surfactant, and pH buffering may be a potential disinfectant for the nursery industry (Peters, 2012). After testing and implementation in both medical and military industries, scientists at SRO believe that E-B may be a valid disinfectant option for nursery plants (Peters, 2012). Since E-B has never been tested on plant material,

research needs to be completed to determine any phytotoxic effects. In this research, Electro-BioCide will be tested alongside Oxidate (BioSafe Systems, East Hartford, CT), a widely used hydrogen dioxide disinfectant, to determine if it may replace Oxidate as a safer broad-spectrum oxidant disinfectant.

To enhance our understanding of any impacts E-B may have on camellia foliage, chlorophyll fluorescence was used to determine foliar damage. Chlorophyll fluorescence has proven on many occasions to be useful in detecting stress in plants from various biotic and abiotic stressors, including chemical damage, even before visual indicators are present (Baker, 2008; Barbagallo et al., 2003; Krugh et al., 1996; Leipner, 2011; Maxwell et al., 2000; Percival, 2005; Xia et al., 2006).

It is necessary that growers reduce mortality during nursery production and prevent transport of exotic pathogens, such as *P. ramorum*. To achieve this, information is needed on valid methods for disinfectant treatments of nursery plants. Before being used to disinfect or disinfest plant material, any disinfectant needs to be evaluated to determine its toxicity to plants. This research attempted to answer the following objectives:

- Evaluate phytotoxicity of various E-B concentrations to camellia.
- Compare these findings with phytotoxicity ratings of a common industry disinfectant.
- Determine if the number of consecutive spray applications impacts the physical and or photosynthetic health of the plant.
- Determine the common characteristics of physical injury on Camellia from E-B.
- Determine the impact of a surfactant in disinfectant-induced phytotoxicity.

LITERATURE REVIEW

Phytotoxicity of Plant Material to Oxidant Foliar Treatments

Phytotoxicity is the negative response of plant material to an applied chemical or compound. It may be the desired result in the case of applying herbicides to eradicate weeds or it may be the undesired consequence of a necessary pesticide application. In either case it is important to know the threshold of the compound being applied, which will result in the desired plant reaction. Due to the importance and relatively small window for error there has been extensive research on plant phytotoxicity to a variety of chemicals.

Unfortunately, many compounds that effectively eradicate plant pest problems also have detrimental effects to plant health. As a result, chemicals whose efficacy concentration needed to kill plant pathogens is lower than that which cause phytotoxicity need to be found and utilized. Carillo et al. (1996) determined that Halox E-100, a commercial chlorine dioxide solution (sodium chlorite), did not tend to have significant toxic effects until greater than 200 ppm was applied five times at three day intervals to radish and lettuce seedlings. Therefore, if a pathogen only required a 100 ppm ClO_2 treatment for eradication, Halox E-100 would be an acceptable treatment option.

Following the above mentioned study, Copes et al. (2003) investigated the impact of chlorine dioxide (ClO_2 - 25% sodium chlorite, 1 to 4.5% sodium chloride) on several nursery plants. In this study, eight bedding plant and nine shrub species were sprayed with regular and excessive rates of chlorine dioxide, five times, at 3-day intervals, to determine if plant damage would result. Six separate experiments were completed, four which contained ClO_2, and two

which contained hydrogen dioxide (H_2O_2 – 14.4% w/w/ hydrochloric acid) treatments. In experiment one, 0, 2, 20, 200, and 2,000 ppm ClO_2 concentrations were used. In additional experiments, 0, 5, 50, 100, and 1,000 ppm ClO_2 concentrations were used. Rates greater than 100 ppm were considered excessive and were used to test the risk of potential damage. Copes et al. (2003) determined that for flowers and leaves of all plants, <4% toxicity occurred at 2, 5, and 20 ppm ClO_2, indicating an insignificant amount of damage. At 50 and 100 ppm ClO_2, ratings of >4% did not occur until after four to five applications for most species. 1,000 and 2,000 ppm ClO_2 damaged all plant species after between 1 and 5 applications depending on the plant species (Copes et al., 2003).

Two H_2O_2 experiments, using five bedding plant and three shrub species, were also completed by Copes et al. (2003). H_2O_2 concentrations of 0, 900, 2,700, 5,400, and 10,200 ppm were used. 5,400 and 10,200 ppm were used as excessive rates to test the risk of potential damage. Rates of 900 and 2,700 ppm H_2O_2 did not damage most plants. In addition, 5,400 ppm H_2O_2 did not damage most plants if applied fewer than four consecutive times.

Phytotoxicity symptoms associated with this study included necrotic leaf tips and margins, necrotic leaf spots and blotches, and leaf death (Copes et al., 2003). Similarly, Cayanan et al. (2008) found foliar necrotic mottling, necrosis, chlorosis, decreased plant height, and increased premature leaf abscission to be common phytotoxic effects of treating five container-grown nursery species with free chlorine (sodium hypochlorite) in overhead irrigation water. While these effects may be detrimental to the plant or its marketability, minor phytotoxic symptoms may go unnoticed. It is important to evaluate both the type and amount of damage

caused by a compound to determine its phytotoxicity threshold, keeping in mind that this effect will vary greatly depending on the plant species being treated.

Electro-BioCide

Electro-BioCide manufactured by SRO (Denver, CO) is a patent-protected, EPA registered (87492-1), general use oxidant disinfectant, composed of a proprietary blend of chlorine dioxide (ClO_2), surfactant, and pH buffering (Peters, 2012). This important breakthrough in solution chemistry is capable of surpassing many of the shortcomings of conventional chlorine dioxide solutions. Some of these limitations include: high toxicity to humans and animals, having a limited shelf-life, being highly corrosive to equipment and surfaces, and limited efficacy (Peters, 2012). Electro-BioCide overcomes these impediments with a formulation that is strategically designed to limit these problems. Electro-BioCide is an EPA category IV, or Practically Non-Toxic, solution (SRO, 2012). Rather than the typical shelf-life of hours to about two days, Electro-Biocide remains stable longer than 60 days and has tested to be compatible with most common surfaces (Peters, 2012).

One major benefit of Electro-BioCide is the ability to generate the product on-site using local water supplies (Peters, 2012). This allows any quantity of solution to be prepared upon demand, rather than the conventional method of mixing, shipping, and storing large quantities of ClO_2 products. The benefits remain simple in that the exact amount of solution needed can be mixed on site, resulting in a fresher more active product, with less waste and generation of harmful by-products, and an overall smaller industrial footprint.

In addition to the oxidizing capabilities of ClO_2, E-B also contains surfactants, which may improve the solutions overall effectiveness. The goal in adding surfactant to a solution is two-fold. First, surfactants are used to facilitate penetration of pathogen protective structures, such that the oxidant can reach the pathogen and kill it. Current research does little to support this theory and often reveals solutions with and without surfactant resulting in similar efficacy (Keskinen et al., 2011). The second goal behind the use of surfactants in oxidant solutions is to increase the amount of surface space covered and active time on the leaf surface. This may result in increased pathogen mortality due to increased chemical exposure time (Keskinen et al., 2011).

Comparison to H_2O_2

Another product, hydrogen dioxide (Oxidate®, BioSafe Systems, East Hartford, CT), is EPA approved (#70299-2) and widely applied for use against a variety of plant pathogens including wilts, mildews, *Pythium*, and *Phytopthtora* (BioSafe Systems, 2008; Nuñez-Palenius et al., 2012). Oxidate has been used to effectively reduce the impacts of bacteria, fungi, and algae on contact (Kleczewski et al., 2011). OxiDate is a 27% hydrogen dioxide (H_2O_2) and 2% peroxyacetic acid solution that when diluted to the desired concentration, is labeled for use on over 25 crops including fruits, vegetables, nuts, and mushrooms (BioSafe Systems, 2008).

In contrast to Electro-BioCide, OxiDate is known to have similar problems to many other products on the market. OxiDate is considered to be corrosive and dangerously reactive (BioSafe Systems, 2008). While OxiDate is considered to be toxic to simple cell and aquatic

organisms, its danger to the environment is considered to be limited, when used according to the label (BioSafe Systems, 2008).

Use of Chlorine Dioxide Solutions Against Common Pathogens

Chlorine dioxide is unique in that it obtains its gaseous state when suspended in water (U.S. EPA, 1999). Unlike hydrochlorites and chlorine gas, which quickly hydrolyze to form hypochlorous acid in water, ClO_2 can be mixed in significantly higher concentrations and remains stable for longer periods of time (Copes et al., 2004). These characteristics enhance the ability of ClO_2 to disinfest many common pathogenic microorganisms.

Previous research reveals that ClO_2 is greatly beneficial in reducing pathogen infestations on plants. In a study evaluating the effects of pH and oxidation reduction potential (ORP) on solution effectiveness in vitro, ClO_2 (15% sodium chlorite, 4.5% sodium chloride) solutions resulted in a LD_{50} kill of *Thielaviopsis* and *Fusarium* conidia at concentrations between 0.5 mg/L ClO_2 and 11.9mg/L ClO_2 (Copes et al., 2004). When freshly cut leaves of Romaine lettuce were treated with 100 ppm ClO_2 (sodium hypochlorite) with and without two surfactant detergents, a 6.86-6.96 log CFU/g kill for *Escherichia coli* O157:H7 was obtained (Keskinen et al., 2011). In a similar study on fresh-cut Red Chard, ClO_2 was able to substantially prevent *E. coli* O157:H7 from cross-contaminating non-infested chard, although, it was not as effective at preventing the spread of *Salmonella* (Tomas-Callejas et al., 2012). In addition to treatment of mature plant material, 100 µL/mL ClO_2 (hydrochloric acid and sodium chlorite solution) applications may also be used to effectively prevent *Cronobacter* spp. on radish seeds (Kim et al., 2013). While *E. coli*, *Salmonella*, and *Cronobacter* are human bacterial pathogens, which are

transmitted to humans on infected plant material, these studies give insight into the potential of ClO_2 products to disinfect plant material for fungal plant pathogens.

pH and ORP of Oxidant Solutions

A variety of studies have been completed within the last 10 years, which examine the effectiveness of chlorine as a disinfectant for various plant pathogens under varying pH and ORP conditions. Copes et al. (2004) examined the impact inorganic ions (ammonium, nitrate, copper, iron, manganese, and zinc) and pH (5 and 8) have on the biocidal activity of ClO_2 (15% sodium chlorite and 4.5% sodium chloride). To accomplish this research, Copes et al. (2004) used ClO_2 solutions at 11 concentrations mixed with nitrogen and hard water solution at pH 5 and 8 (Copes et al., 2004). They evaluated solutions on efficacy based on the lethal dose resulting in 50% mortality (LD_{50}) of *Thielaviopsis basicola* and *Fusarium oxysporum* spores. Copes et al. (2004) found that higher concentrations of ClO_2 were needed at pH 8 to achieve the same efficacy as lower concentrations at pH 5. Introduction of the divalent metal ion solution (Cu, Fe, Mn, and Zn solution) created a demand for higher ClO_2 concentrations to maintain an LD_{50} kill.

In a later study, Lang et al. (2008) monitored the mortality of *Phythium* zoospores in chlorinated water using oxidation reduction potential (ORP). They argued that using ORP "is a reliable, real-time measurement of the oxidizing potential of a chlorine solution," compared to a dose rate (Lang et al., 2008). Lang et al. (2008) tested the impacts of raising the ORP of municipal water, containing two species of *Phythium* zoospores, through adding 0.125, 0.5 and 2 mg/L of chlorine (sodium hypochlorite), both with no change to water pH and when water pH

was lowered to 6 prior to the addition of chlorine. Results indicated when pH is lowered to 6, prior to the addition of chlorine, 100 percent of *Phythium* zoospores were killed after 30 seconds exposure to 0.5 mg/L chlorine, at an ORP between 748 and 790 mV. On the other hand, some zoospores survived after four minutes of exposure to 2 mg/L chlorine, in water where the pH had not been previously lowered, and the ORP was at 790 mV. This indicates that lowering the initial pH of municipal water, prior to the addition of chlorine, enhances efficacy, such that a lower concentration of chlorine may be used to accomplish the same mortality rate.

Similar to these results, Suslow (2004) found that at an ORP of 650 to 700 mV *E. coli* O157:H7 and *Salmonella* are killed at less than 30 seconds exposure. In agreement with Lang et al. (2008), Suslow (2004) reported that lowering a solutions pH increases the amount of hypochlorous acid (HOCl) in the solution, thus increasing the solutions ORP. Therefore, the efficacy potential of a solution with a pH of 6.5 and an ORP of 700 mV will be the same as a solution with a pH of 8.5 and an ORP of 700 mV, but it would require much higher concentrations of hypochlorite to achieve that ORP at pH 8.5 (Suslow, 2004). As a result, at a lower pH, less chemical (hypochlorite) can be used to achieve the same efficacy results.

These dynamics found in Copes et al. (2004), Lang et al. (2008), and Suslow (2004) can be used when generating Electro-BioCide solutions. Since the process of oxidization can lead to a decrease in disinfestation properties, due to a breakdown of hypochlorous acid into hypochlorite when chlorine dissolves in water, the oxidant concentration needs to be closely monitored (Lang et al., 2008). By decreasing the solutions pH prior to the addition of ClO_2 the

solution may become a more effective biocide on tough plant pathogens, such as *Phytophthora ramorum*, and other oomycetes (Lang et al., 2008).

Phytophthora ramorum

Sudden Oak Death, caused by the exotic plant pathogen, *Phytophthora ramorum*, is a disease impacting native trees and perennial herbaceous and woody ornamentals in coastal areas of California, Oregon, and Washington as well as many European countries. *P. ramorum* "causes trunk cankers and widespread mortality on tanoak (*Lithocarpus densiflorus*), and oak (*Quercus* spp.)" in addition to "leafspots and blights on numerous other native hosts in California and Oregon woodlands," such as camellia and rhododendron (Frankel, 2008; Tjosvold, 2008). In the western United States, this pathogen has proven to be a costly invasive disease since its detection in California in the 1990s. It is being spread currently between nurseries and surrounding natural areas through infected and infested plant material, soil, water and mechanical transmission (Frankel, 2008; Grunwald et al., 2008; Tubajika et al., 2006).

P. ramorum is an Oomycota in the family Pythiaceae (Grunwald et al., 2008). The production of characteristic oospores and terminal chlamydospores makes *P. ramorum* especially difficult to eradicate, due to these survival structure's abilities to persist for long periods in and on infected soil and plant material (Davidson et al., 2003). *Phytophthora* spores are easily dispersed mechanically as well as by wind and water (Grunwald et al., 2008). There are currently 137 known host species, including 45 species which are being regulated by USDA-APHIS as of January 2012. This wide host range results in rapid dissemination of the pathogen during ideal environmental conditions (USDA-APHIS, 2013).

To prevent the spread of *P. ramorum* to uninfected regions, the United States Department of Agriculture (USDA) Animal Plant Health Inspection Service (APHIS) Plant Protection and Quarantine (PPQ) has been active in developing preventative strategies. Currently, 15 counties across the coastal regions of California and Oregon have *P. ramorum* quarantines, in addition to regulations, which were placed on the entire state of California, Oregon, and Washington (USDA-APHIS, 2013). Current efforts attempt to contain the disease in California woodlands, eradicate the disease in Oregon woodlands and nurseries, and quarantine the disease in infected areas around the world (Grunwald et al., 2008). In the United States, these efforts are intended to reduce the tens of millions of dollars in losses estimated in 2008 as a result of direct and indirect costs of *P. ramorum* (Grunwald et al., 2008).

Chlorophyll Fluorescence

Chlorophyll fluorescence is a useful tool in measuring plant stress and it may be useful to detect damage from chemicals, such as disinfectants (Krugh et al., 1996; Percival, 2005; Xia et al., 2006). It has been shown on multiple occasions that fluorescence is an important indicator of plant stress (Baker, 2008; Leipner, 2011; Maxwell et al., 2000). Chlorophyll fluorescence imaging is known to be able to detect changes in plant metabolism as a result of chemical treatments before any visual effects are present (Barbagallo et al., 2003). Decreases in maximum quantum efficiency of PSII photochemistry (Fv/Fm) are so commonly observed when plants are exposed to abiotic and biotic stresses that these fluorescence measurements have become “a simple and rapid way of monitoring stress,” (Baker, 2008).

Chlorophyll fluorescence functions by monitoring the efficiency of the light reactions of photosynthesis enclosed within a single leaf cuvette, which can then be used to make assumptions about the whole plant. In Photosystem II (PSII) electrons from water molecules in the thylakoid lumen are excited by light energy and given to the primary electron acceptor (Taiz et al., 2006). In a healthy plant, plastoquinone then donates these electrons to the cytochrome b complex, while the protons from the water are discharged to the lumen. Plastocyanin then donates these electrons to Photosystem I, where NADPH is made (Taiz et al., 2006). Unfortunately, this system does not always function as such.

When light energy enters the chlorophyll of a plant, the electrons may undergo one of three fates. First, the electrons may be transported through the electron transport chain as described above. Alternatively, these electrons may also be dissipated as either heat or fluorescence (Garcia et al., 2012). Assuming these are the only three fates of electron light energy entering a plant, by measuring any two of the factors assumptions can be made about the third. Chlorophyll fluorescence measurements, using the LiCOR 6400XT leaf chamber fluorometer, measure heat and fluorescence thus gaining insight into the number of electrons being effectively used in plant photochemistry to make NADPH (Garcia et al., 2012).

These measurements can be done on either dark-adapted or light-adapted plant material depending on the desired information. When a plant is in a dark-adapted state, or the leaf is kept in complete darkness, the primary quinone electron acceptor of PSII (Q_A) becomes maximally oxidized and is capable of receiving electrons from P680 (Baker, 2008). When exposed to a weak light pulse, the minimal level of florescence (F_o) can be measured. Exposing

the same leaf to higher photosynthetically active photon flux density will result in Q_A becoming maximally reduced, revealing the maximum fluorescence level (F_m). Maximum quantum yield of Q_A reduction (Fv/Fm) is a ratio of $\frac{F_m - F_0}{F_m}$ (Fig. 1). Across 44 species of healthy vascular plants, Fv/Fm remains remarkably constant, around 0.83, or 83% of electrons are being used to make NADPH (Bjorkman et al., 1987). Often, decreases in Fv/Fm are observed when plants are exposed to both biotic and abiotic stressors (Baker, 2008). This decrease is usually caused by a simultaneous decrease in F_m and increase in F_o (Leipner, 2011).

$Fv/Fm = \frac{F_m - F_0}{F_m}$	$\theta_{PSII} = \frac{F' - F_m'}{F_m'}$.
Dark-Adapted Fluorescence	Light-Adapted Fluorescence

Figure 1. Dark- and Light-Adapted Fluorescence Measurement Parameters (Baker, 2008).

As an example, chlorophyll fluorescence has been used to detect pesticide sensitivity in vascular plants on several occasions. When five nonherbicidal pesticides were applied to Mung beans (*Phaseolus vulgaris*) two of the chemicals decreased the Fv/Fm values (Krugh et al., 1996). Similarly, when nine pesticides were applied to *Cucumis sativus* foliage, one pesticide (1,1'-dimethyl-4,4'-bipyridinium dichloride) resulted in detrimental decreases in Fv/Fm one day later, while three others had smaller decreases, and the remaining five had no effect (Xia et al., 2006). These data reveal that chlorophyll fluorescence is able to differentiate the amount of stress being placed on the photosynthetic apparatus by various chemical treatments.

The alternative to dark-adapted maximum quantum yield fluorescence analysis is light-adapted PSII operating efficiency (θ_{PSII} or F_q'/F_m' or $\Delta F/F_m'$) (Baker, 2008). θ PSII measurements are taken on plant material that has remained in actinic light. After receiving a pulse of light, the plant fluorescence at actinic light levels (F') rises to its maximal fluorescence level (F_m'). $\theta_{PSII} = \frac{F' - F_{m'}}{F_{m'}}$ (Fig. 1). θ_{PSII} reveals the PSII operating efficiency under varying environmental conditions rather than the steady environment provided during Fv/Fm measurements (Baker, 2008). Unfortunately, there are several drawbacks to using θ PSII measurements including: an inability to get accurate F_o measurements at wavelengths above 700 nm and an overestimation of F_m' if saturating light pulses induce plastoquinol reduction in addition to plastoquinone reduction (Baker, 2008).

With these drawbacks in mind, it is important to note that θ_{PSII} is also a good method for detecting plant stress. As mentioned before, when nine pesticides were applied to *Cucumis sativus* foliage, differences in Fv/Fm were detected between treatments (Xia et al., 2006). Similarly, changes in θ_{PSII} were also detectable between treatments. Interestingly, dark-adapted fluorescence measurements detected significant decreases in Fv/Fm from four of the pesticide treatments (bipyridinium dichloride, imidacloprid, chlorpyrifos, and abamectin), while θ_{PSII} detected significant decreases from three of the pesticide treatments (bipyridinium dichloride, fluazifop-*p*-butyl, and chlorpyrifos). These data suggest that measuring both Fv/Fm and θ_{PSII} may be ideal in order to understand more of how the plant is reacting under these stress conditions.

METHOD AND MATERIALS

Study design

This study was conducted in January 2013, in a USDA Agricultural Research Service greenhouse in Fort Collins, CO (40.585° N, 105.084° W). The study was designed to be completely randomized, with four replications within a treatment. The ten disinfectant treatments were divided into two groups of five treatments each (Table 1). The treatments were grouped because only 20 plants could be measured for fluorescence in a given day. Thus the twenty plants per group were treated and measured on sequential days to maintain the same time intervals of measurement for all the treatments. The incomplete factorial design included three study factors: disinfectant type, disinfectant concentration, and surfactant concentration.

Table 1. Disinfectant treatments and the number of replicate camellia plants treated.

Disinfectant Treatment	Number of Replicate Camellias
	Group A
0 $mg \cdot L^{-1}$ (Water)	4
0 $mg \cdot L^{-1}$ + Sarc (Water + Sarc)	4
100 $mg \cdot L^{-1}$ ClO_2	4
200 $mg \cdot L^{-1}$ ClO_2	4
400 $mg \cdot L^{-1}$ ClO_2	4
	Group B
100 $mg \cdot L^{-1}$ H_2O_2	4
100 $mg \cdot L^{-1}$ H_2O_2 + Sarc	4
100 $mg \cdot L^{-1}$ ClO_2 + Sarc (E-B)	4
200 $mg \cdot L^{-1}$ ClO_2 + Sarc (E-B)	4
400 $mg \cdot L^{-1}$ ClO_2 + Sarc (E-B	4
Total	40

Visual injury ratings and maximum quantum efficiency of PSII (Fv/Fm) were measured over seven time periods. Three environmental cofactors were included in the fluorescent test including: soil temperature, soil moisture, and leaf temperature. Two oxidant properties were measured and included in the analyses including pH and oxidation reduction potential (ORP).

Plant Material

The specimens used for this study were *Camellia japonica* 'Scentsation.' This cultivar is a semi-dwarf evergreen shrub with 45 cm to 60 cm tall foliage. 55 camellia plants, in 5 gallon nursery containers, were supplied by Hines Nursery in Winters, CA and delivered on December 10, 2012.

Forty camellia plants were randomly assigned to treatment groups using the random assortment generator in JMP software (SAS Institute Inc., Cary, NC). Plants were labeled with the corresponding treatment and replicate number from the randomly generated list.

Plants were watered as needed with 200 $mg \cdot L^{-1}$ 20-10-20 ($N\text{-}P_2O_5\text{-}K_2O$) constant feed Jack's water soluble fertilizer (J.R Peters Inc., Allentown, PA). Supplemental high pressure sodium lighting was used to encourage vegetative growth, nightly from 2300 to 0200. On December 13, 2012 the Camellias were treated with granular Imidacloprid, a systemic insecticide, applied to the soil surface at labeled rate, for prophylactic control of whiteflies and aphids.

Disinfectant Treatments

This study consisted of ten disinfectant treatments (Table 1). The two oxidants used in the solutions were chlorine dioxide (ClO_2) and hydrogen dioxide (H_2O_2). Electro-BioCide (E-B) is an EPA registered (87492-1), general use disinfectant composed of a proprietary blend of ClO_2, sarcosinate surfactant, and pH buffering additive (Strategic Resource Optimization, Denver, CO). ClO_2 was applied at three rates (100, 200, and 400 $mg \cdot L^{-1}$), with (0.2%) and without (0%) sarcosinate surfactant. Oxidate is a H_2O_2 disinfectant that is EPA registered (70299-2) for use on row crops and nursery plants (Biosafe Systems, Glastonbury, CT). Oxidate was used as an industry standard, applied only at label rate (100 $mg \cdot L^{-1}$), with (0.2%) and without (0%) sarcosinate surfactant, to compare effects to those of the ClO_2 treatments.

Treatments were divided into “Group A” and “Group B,” so that measurements could be taken equal distance after application. Foliar spray applications, visual analysis, and Fv/Fm measurements were taken at three day intervals (Table 2). Five foliar spray applications and seven visual and Fv/Fm assessments were completed. The five treatments in Group A were foliar sprayed, visually analyzed, and measured for Fv/Fm on the same days. The day following each of these procedures the foliar application, visual analysis, and Fv/Fm measurements were taken on Group B (Table 2).

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
		1	2 Spray A Dark Adapt in Basement	3 Spray B Fv/Fm A Dark Adapt in Basement	4 Fv/Fm B	5 Visual A Spray A Dark Adapt in Basement
6 Visual B Spray B Fv/Fm A Dark Adapt in Basement	7 Fv/Fm B	8 Visual A Spray A Dark Adapt in Basement	9 Visual B Spray B Fv/Fm A Dark Adapt in Basement	10 Fv/Fm B	11 Visual A Spray A Dark Adapt in Basement	12 Visual B Spray B Fv/Fm A Dark Adapt in Basement
13 Fv/Fm B	14 Visual A Spray A Dark Adapt in Basement	15 Visual B Spray B Fv/Fm A Dark Adapt in Basement	16 Fv/Fm B	17 Visual A Dark Adapt in Basement	18 Visual B Fv/Fm A Dark Adapt in Basement	19 Fv/Fm B
20 Visual A Dark Adapt in Basement	21 Visual B Fv/Fm A Dark Adapt in Basement	22 Fv/Fm B	23	24	25	26
27	28 Visual A	29 Visual B	30	31		

Table 2. Project calendar

pH and ORP Measurements

Fresh ClO_2 and ClO_2 + Sarc was received from SRO for each of the spray dates to ensure no loss in oxidant activity over the five application dates. Additionally, two liters of each Oxidate treatment solution were prepared from concentrate for each of the five application dates. The pH and ORP were measured for each treatment, prior to each of the five applications, to ensure the batches from each application were consistent. Measurements were taken with an Orion 3 Star Plus meter (Total Temperature Instrumentation, Inc., Burlington, VT), proceeding spray application.

Foliar Disinfectant Treatment Application

Beginning on January 2, 2013, plants were repeat sprayed, every three days for five separate applications. Each plant was separated from the rest to prevent drift. The disinfection application was made using a negatively charged electrostatic sprayer (Electrostatic Spraying Systems Inc., Watkinsville, GA). A stopwatch was used to regulate the spray time for each plant to 40 seconds. Using a revolving table top, plants were continuously spun in circles while the upper and lower surface of the foliage was sprayed for 20 seconds each. Approximately 40 mL of liquid disinfectant solution was applied to each plant to allow saturation, but not liquid runoff from the leaf. The spray lines were purged between each change in spray treatment, but not between replicates. After being sprayed, each plant was moved away from the spray application area to dry before being placed in complete darkness overnight for dark-adapted fluorescence measurements. Most plants were dry to touch within thirty minutes.

Visual Assessment

A detailed description of the visual assessment and rating scale used in this study can be found in Copes et al. (2003). Visual assessments were taken three days after each of the five spray applications, and again six and fourteen days following the final spray application. Ratings were taken earlier in the day prior to each consecutive spray application. Visual assessments began with two researchers evaluating foliar injury symptoms on each plant, using the Barrat-Horsfall scale. After each researcher had assessed the twenty plants being evaluated on that day, the two assessment scores were compared. If there were discrepancies between the two assessment scores the plant in question was revisited and discussed until the evaluators agreed upon a score.

Fluorescence Measurements

Dark-adapted fluorescence measurements were taken the day following each disinfectant spray application. Additionally, fluorescence measurements were taken four and seven days following the final spray application. On each plant, the four youngest, fully expanded leaves, on January 1, 2013, were chosen for fluorescence measurements. Maximum operating efficiency of photosystem II (Fv/Fm) fluorescence measurements were taken with a LI-COR 6400XT, fitted with a leaf chamber fluorometer. Measurements were taken between 0730 and 1400 on each of the measurement dates to avoid changes in photosynthesis due to circadian rhythm (C. Ramsey, unpublished data).

Fluorescence measurements were taken on foliage that had been in complete darkness, or dark-adapted, in the greenhouse headhouse basement for a minimum of ten hours. Dark

adaption was used to shut down photosynthesis in plants, putting them in a resting state. Since it would not activate photosynthesis and interfere with the Fv/Fm measurements, a UV-A black-light was used to illuminate the workspace.

During the first round of fluorescence measurements (January 3 and 4, 2013) after clamping the LI-COR 6400XT on each leaf, the perimeter of the leaf chamber was outlined onto the leaf, using a white paint pen. This was done on the four leaves of each plant so that repeated measurements could be taken at the same sampling spot on each measurement date.

During each fluorescence measurement, the system was allowed to stabilize until dF/dt was between -5 and 5 before a square flash measurement was taken.

Soil Moisture and Temperature

A Decagon soil moisture data logger and 5 cm soil sensor (ECH_2O-EC-5, Decagon Devices, Pullman, WA) was used to collect soil moisture and temperature during each fluorescence measurement. Soil moisture and temperature measurements were recorded each time a fluorescence measurement was taken. The soil measurements were taken half way between the edge of the pot and the plant stem, about 5 cm below the soil surface.

Statistical Analysis

An alpha of 0.05 was chosen prior to data analysis to determine statistical significance. Maximum quantum efficiency fluorescence measurements (Fv/Fm), and visual assessment data were analyzed as a repeated measures test over the seven measurement dates. The pH, ORP, soil moisture, soil temperature, and leaf temperature data were included as covariates in each analysis to test for their interactions with the study factors. The visual assessment data were

analyzed with a logistical regression test, while the Fv/Fm data were analyzed using a maximum likelihood test (REML). Insignificant study factors and interactions were dropped from the final test models. Regression analysis was used to test the relationships between soil moisture, soil temperature, and Fv/Fm.

Since pH and ORP were measured on five measurement intervals, while visual and Fv/Fm data were measured on seven measurement intervals (spaced at three days), data were analyzed separately. The first analysis was run over seven measurement intervals and did not include pH and ORP data in the model. The second analysis was run over five measurement intervals and examined the effects of pH and ORP on treatment differences.

RESULTS AND DISCUSSION

RESULTS ANALYSIS 1: VISUAL AND FLUORESCENCE DATA OVER SEVEN MEASUREMENT INTERVALS EXCLUDING pH AND ORP MEASURES

Both the visual and fluorescence data were taken at seven measurement intervals - five measurements upon receipt and application of the new batches and two additional measurements following the final application. The additional two measurements were used to evaluate any lasting effects four and seven days following the final spray application. Conversely, pH and ORP measures were taken only when each new batch of disinfectant solutions was mixed. Therefore, pH and ORP data was taken only five times. As a result, pH and ORP data can only be run against the visual and fluorescence data for the first five measurement intervals. The following data represents findings when pH and ORP were not included in the analysis, thus that visual and fluorescence data can be analyzed over the seven measurement intervals. Results including pH and ORP data will be presented later in the discussion (Analysis 2).

Visual Assessment

Results represent the estimate of running a logistical regression on the data (Prob. ChiSq = <0.0001, RSquare (U) = 0.70) (Appendix A). The logistical regression resulted in the following interactions: oxidant and sarcosinate concentration, oxidant and measurement interval, sarcosinate concentration and oxidant concentration, measurement interval and oxidant

concentration, and oxidant concentration and mean soil temperature. Data were an average of the four replicate plants in each treatment group.

Plants in all ten treatments had no damage at measurement interval one (Fig. 2, A). The two water controls (0 $mg \cdot L^{-1}$ oxidant concentration) responded very similarly throughout the seven measurement intervals. The water treatment has a lower probability of receiving a high visual rating than the water + Sarc treatment in all measurement intervals. The water + Sarc treatment has a 50% decline in mean probability of receiving a visual rating of one between measurement intervals two and three. On the contrary, the water treatment had a decline of less than 5% between measurement interval two and three. Furthermore, there was an 85% increase in the probability of the water treatment to receive a mean visual score of two, compared to a 24% increase in the water + Sarc treatment from measurement interval five to measurement interval seven.

At 100 $mg \cdot L^{-1}$ oxidant concentration, both the ClO_2 and ClO_2 + Sarc treatments responded very similarly in regards to visual injury across the seven measurement intervals (Fig. 2, B). Across the seven measurement intervals, the 100 $mg \cdot L^{-1}$ ClO_2 + Sarc treatment had a higher mean probability of more visual damage. By measurement interval seven, 100 $mg \cdot L^{-1}$ ClO_2 + Sarc had a nearly 45% higher probability of scoring a three, and broke the marketability threshold of at least 50% of the plants having greater than 3-6% visual injury (Fig. 2, Table 3).

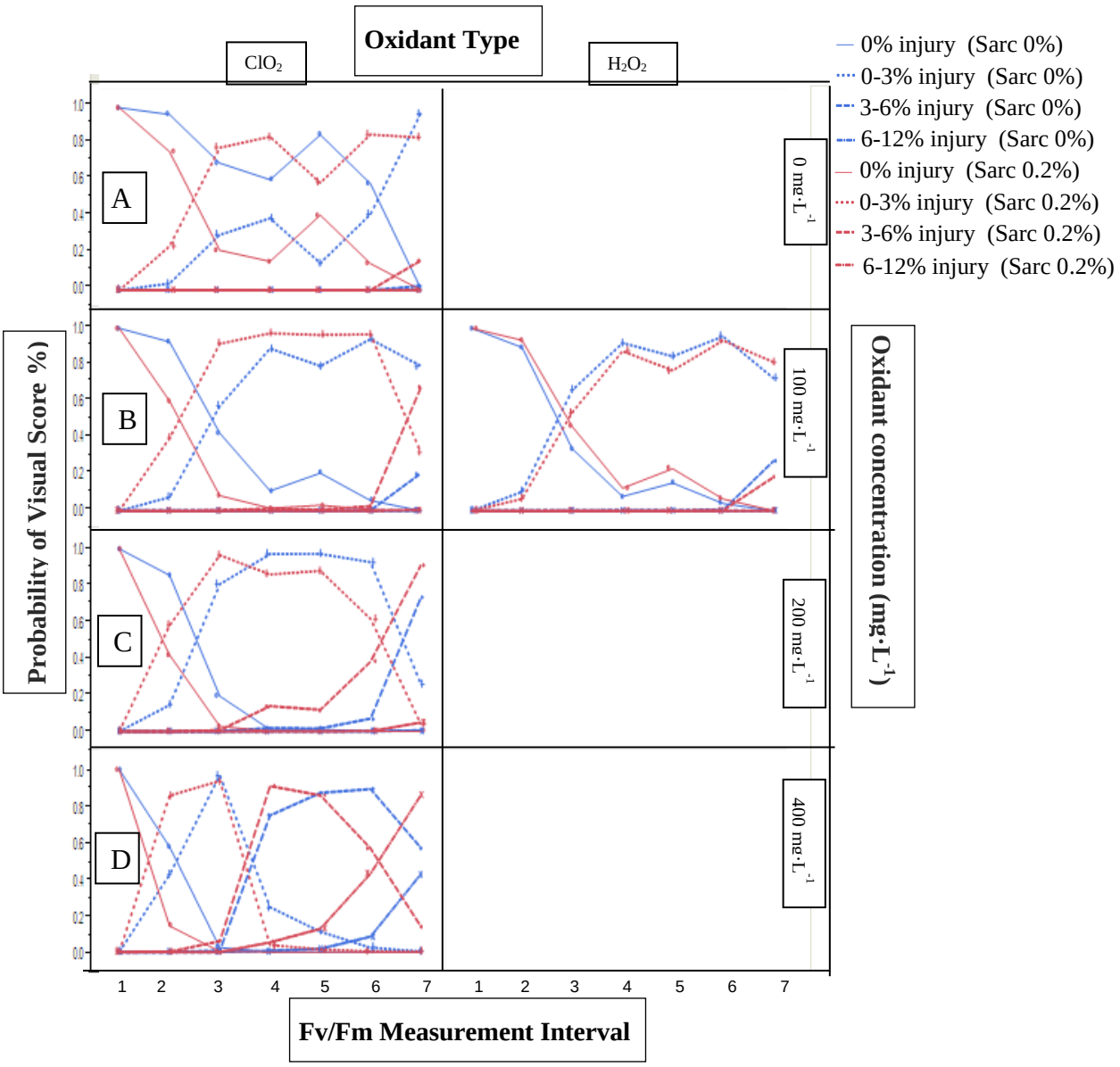

Figure 2. Probability of a camellia plant treated with an oxidant disinfectant to exhibit visual injury damage. Chlorine dioxide oxidant treatments are represented in the left column, while the hydrogen dioxide oxidant is represented on the right. Rows represent oxidant concentration used. Treatments with and without Sarc surfactant are present in the same quadrant, differentiated by color (None = Blue, Sarc = Red). An increase in the probability of a higher visual injury score at each consecutive measurement interval is present across all camellia plants in all treatment groups. n = 4.

At 200 mg·L^{-1} both the ClO_2 and ClO_2 + Sarc treatments had a rapid decline in the probability of receiving a visual score of one, resulting in a 0% probability of a visual score of one by measurement interval four and three, respectively (Fig. 2, C). Across all measurement intervals, the 200 mg·L^{-1} ClO_2 + Sarc treatment was more likely to have a higher mean probability of receiving a high visual injury score. The marketability threshold was broken by both the 200 mg·L^{-1} ClO_2 (75% mean probability of receiving a three) and 200 mg·L^{-1} ClO_2 + Sarc (90% mean probability of receiving a three) treatments (Fig. 2, Table 3).

Table 3. 10 treatment applications and the number of consecutive applications at 3-day intervals where <3-6% or >3-6% of the whole plant foliage had visual injury. NOTE: Interval 6 and 7 were rest periods following the fifth spray application where visual data was collected, but no treatment solution was applied. n = 4.

	<3-6% Visual Injury		>3-6% Visual Injury	
Oxidant	**Rate (ppm)**	**Analysis Interval**	**Rate (ppm)**	**Analysis Interval**
Chlorine Dioxide	0	7	0	
Chlorine Dioxide	0 + SARC	7	0 + SARC	
Chlorine Dioxide	100	7	100	
Chlorine Dioxide	200	6	200	7
Chlorine Dioxide	400	3	400	4
Chlorine Dioxide	100 + SARC	6	100 + SARC	7
Chlorine Dioxide	200 + SARC	4	200 + SARC	5
Chlorine Dioxide	400 + SARC	3	400 + SARC	4
Hydrogen Dioxide	100	7	100	
Hydrogen Dioxide	100 + SARC	7	100 + SARC	

The 400 mg·L^{-1} ClO_2 and ClO_2 + Sarc treatments both had a drastic decline in mean probability of receiving a visual injury score of one between measurement interval one and two, with a decrease from 100% to 55% and 15% for ClO_2 and ClO_2 + Sarc, respectively (Fig. 2, D). This resulted in a 0% mean probability of receiving a visual score of one by measurement interval three for both treatments. The marketability threshold, of 50% of plants having greater than 3-6% visual injury, was broken by measurement interval four for both treatments (Table 3). This resulted in a mean probability of a visual score of four, of 42% and 85%, by measurement interval seven for 400 mg·L^{-1} ClO_2 and ClO_2 + Sarc, respectively.

The mean probability of visual scores for the two 100 mg·L^{-1} hydrogen dioxide solutions, containing and not-containing surfactant, mimicked each other. Unlike the 100 mg·L^{-1} chlorine dioxide solutions, the 100 mg·L^{-1} H_2O_2 + SARC solution had an increased mean probability of receiving lower visual injury scores compared to the 100 mg·L^{-1} H_2O_2 solution (Fig. 2). Overall, the 100 mg·L^{-1} chlorine and hydrogen dioxide solutions had relatively similar mean probability visual injury scores. The exception to this similarity is a 20-35% greater probability of the H_2O_2 solutions to score a one in measurement interval three through five, compared to the ClO_2 solutions and a 40% increase in the mean probability of receiving a score of a three, in measurement interval seven, for the 100 mg·L^{-1} ClO_2 + Sarc solution.

Chlorophyll Fluorescence Assessment

Results represent least squared means estimates corrected for both soil moisture and soil temperature ($P = <0.0001$, $R^2 = 0.38$, RMSE = 0.0056) (Appendix B). The analysis resulted in the following interactions: oxidant concentration and sarcosinate concentration, oxidant type

and measurement interval, and measurement interval and leaf temperature. Data were an average of the four replicate plants in each treatment group.

Across all ten treatments, a very large range in upper and lower confidence intervals was apparent on measurement interval four (Fig. 3). Additionally, a decrease in mean Fv/Fm occurred during measurement interval five (Fig. 3; Fig. 4). In all treatments, the mean Fv/Fm in measurement interval six recovered to the equivalent mean Fv/Fm value in measurement interval three, and continues to increase in measurement interval seven. In comparison to the eight ClO_2 treatments, the two H_2O_2 treatments had a >50% smaller decrease in Fv/Fm during measurement interval five (Fig. 4).

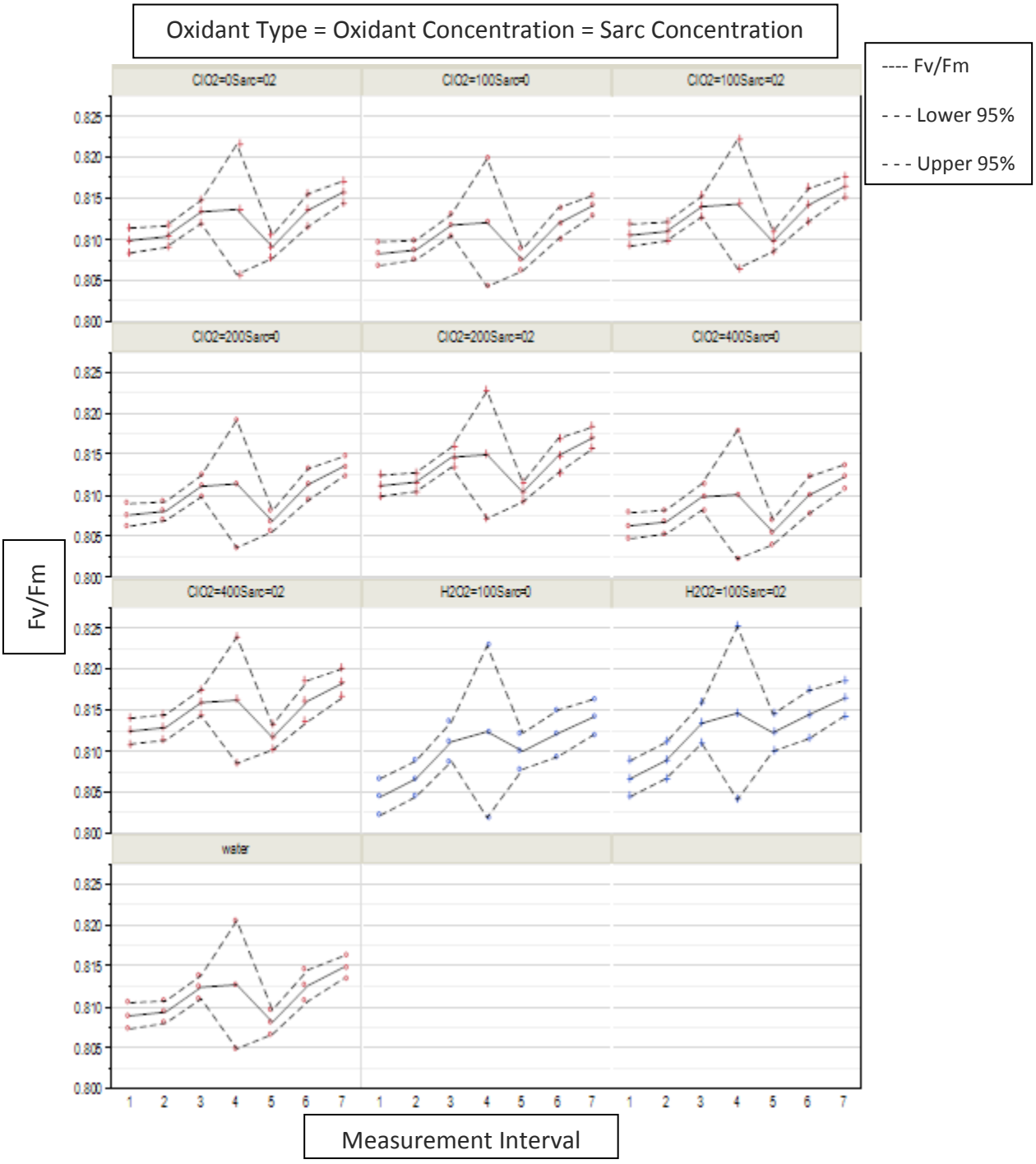

Figure 3. Mean Fv/Fm of camellia plants following application of oxidant treatments taken on seven measurement intervals. Each quadrant represents the effects from one oxidant solution. Dashed lines represent the upper and lower 95% confidence intervals around the mean. Large variance in interval four may be due to a large decrease in temperature during data collection. n = 16.

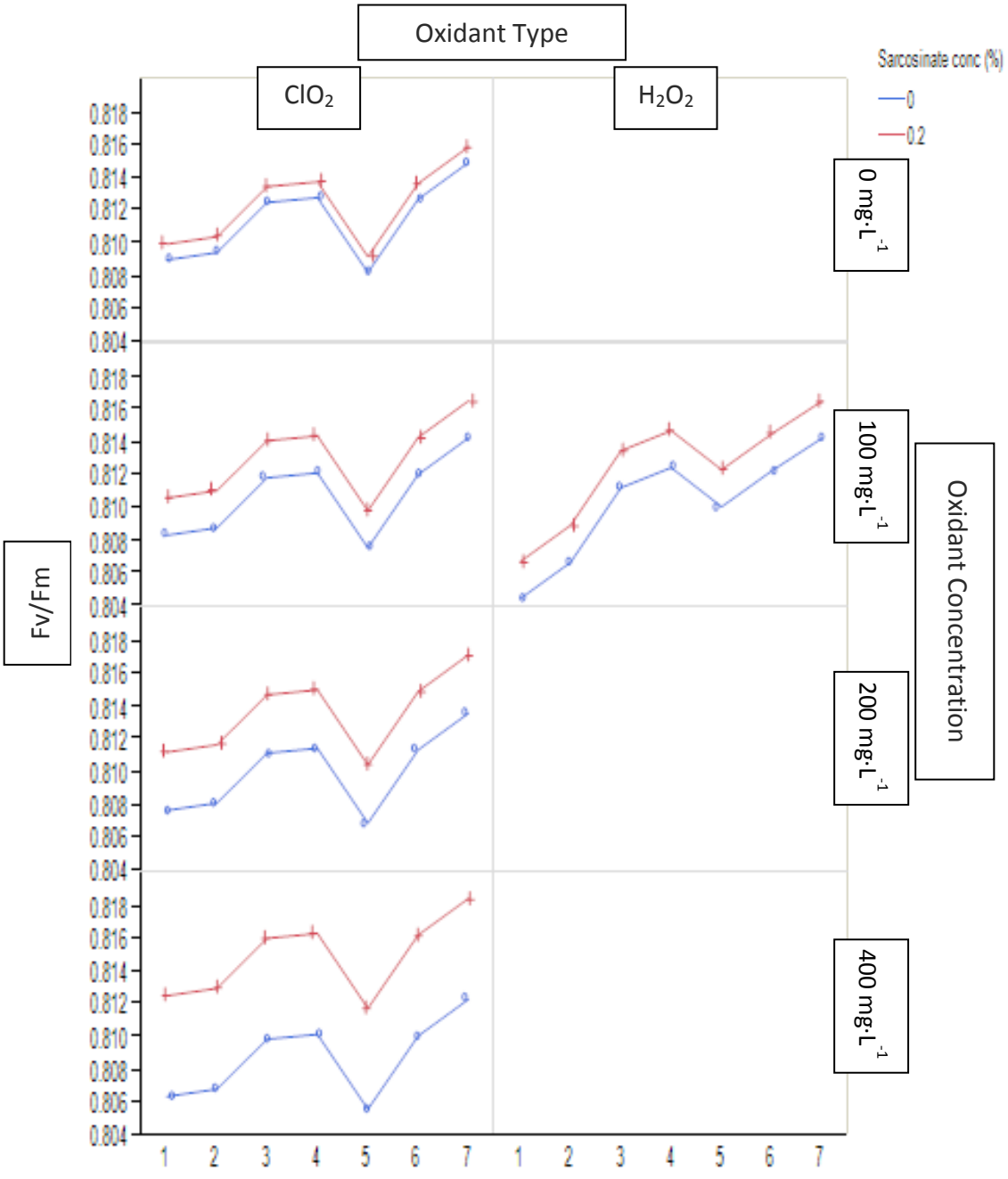

Figure 4. Mean Fv/Fm of camellia plants following application of oxidant treatments taken on seven measurement intervals, comparing treatments with and without Sarc. Chlorine dioxide oxidant treatments are represented in the left column, while the hydrogen dioxide oxidant is represented on the right. Rows represent oxidant concentration used. Treatments with and without Sarc surfactant are present in the same quadrant, differentiated by color (None = Blue, Sarc = Red). n = 16.

At every oxidant concentration, treatments containing sarcosinate surfactant had greater mean Fv/Fm values than the comparable non-surfactant treatments (Fig 4). As oxidant concentration increased, the positive effect of sarcosinate being present became more noticeable (Fig. 4).

Plants in all treatments had an overall net increase in average Fv/Fm between measurement interval one and measurement interval seven (Fig. 4). This increase ranged from a net increase of 0.005 (100 $mg \cdot L^{-1}$ ClO_2 + Sarc) to 0.010 (100 $mg \cdot L^{-1}$ H_2O_2 and 100 $mg \cdot L^{-1}$ H_2O_2 + Sarc), with the typical increase being 0.006 (water, water + SARC, 100, 200 $mg \cdot L^{-1}$, and 400 $mg \cdot L^{-1}$ ClO_2, 200 and 400 $mg \cdot L^{-1}$ ClO_2 + Sarc).

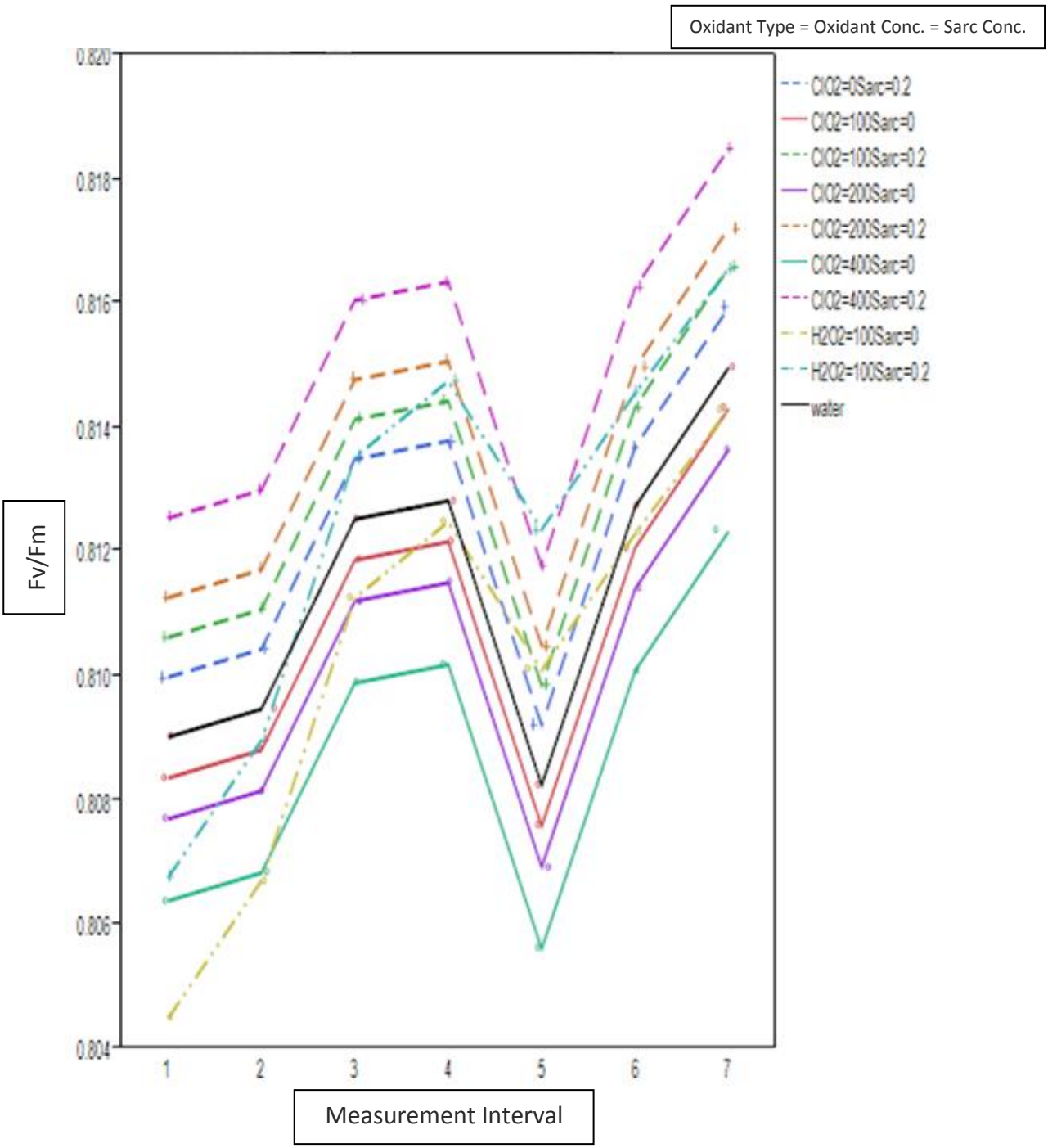

Figure 5. Mean Fv/Fm across the seven measurement intervals for each of the ten treatments, when leaf temperature is controlled in the model across all treatments at 21.76° C, n = 16. At nearly every oxidant concentration, treatments containing sarcosinate surfactant have higher mean Fv/Fm values than the comparable non-surfactant treatments.

DISCUSSION ANALYSIS 1

Visual Assessment

These results indicate that there was an unacceptable visual injury threshold that may be reached given this experimental design and limitations. When ClO_2 and ClO_2 + Sarc are applied to camellia foliage at three day intervals an acceptable marketability threshold lies between 200 and 400 $mg \cdot L^{-1}$ applied between seven and four consecutive times. This threshold may or may not be improved by increasing the time intervals between spray applications and rinsing foliage of excess salt build up (Copes, 2003). In comparison to these results, Copes (2003) found that ClO_2 when applied to two varieties of *Rhododendron* did not result in damage until 20 $mg \cdot L^{-1}$ and 200 $mg \cdot L^{-1}$ were consecutively sprayed four or five times, at three day intervals. Given these results and the results of this project, it is suggested that similar plant material may be treated with a 200 $mg \cdot L^{-1}$ ClO_2 solution up to five times, and potentially up to seven consecutive times, without resulting in excessive visual injury.

Interestingly, results indicated that the 200 and 400 $mg \cdot L^{-1}$ solutions underwent a decline in the probability of receiving a low visual injury rating score by measurement interval three (Fig. 2). These treatments had a 0% probability of receiving a visual injury score of one, with the exception of the 200 $mg \cdot L^{-1}$ ClO_2 solution, which had a mean probability of 19%. This indicated that at higher oxidant concentrations each consecutive treatment application has a more detrimental impact on the plant.

Of particular importance to this research was the impact of sarcosinate surfactant on visual injury ratings. Across the eight chlorine dioxide treatments, the presence of surfactant

increased the probability of a higher mean visual score, with this damage being more severe with increasing time and oxidant concentration. Similarly, Keskinen et al. (2011) found that a 0.4% short chain fatty acid formulation resulted in minor phytotoxicity of Romaine lettuce when submerged in solution for two minutes. One explanation for this increase in visual injury may be a result of the surfactant removing a portion of the leaf cuticle, making it more susceptible to oxidant and environmental damage.

Fascinatingly, between measurement interval five and six, there was a large increase across all ten treatments in the probability of a higher visual injury rating score (Fig. 2). This correlates directly with a drop in Fv/Fm in measurement interval five (Fig. 4). This indicated that Fv/Fm measurements detected a decline in plant health up to three days earlier than visual symptoms appeared. These results correspond directly with the claim that chlorophyll fluorescence is capable of detecting changes in "leaf metabolism considerably before the onset of any visual effects on leaf morphology," (Barbagallo et al., 2003).

Chlorophyll Fluorescence Assessment

Across all ten treatments there was a net increase in average Fv/Fm between measurement interval one and measurement interval seven (Fig. 4). Interestingly, treatments containing the chlorine dioxide oxidant and sarcosinate surfactant had a higher mean Fv/Fm value than the comparable non-surfactant treatments. This is opposite what would have been expected considering the four chlorine dioxide treatments containing surfactant had an increased mean probability of receiving higher visual injury scores. As a result, it must be concluded that the damage caused by the presence of sarcosinate surfactant was purely

cosmetic, and does not detrimentally impact the photosynthetic apparatus, but may in fact enhance it.

Of significant interest to these data, was the dramatic decrease in Fv/Fm of all treatments during measurement interval five (Fig. 4). Easily visible was a very large confidence interval for mean Fv/Fm during measurement interval four of all treatments (Fig. 3). This was caused by a significant drop in average leaf temperature from over 21°C in measurement three and five, to 16.7°C in measurement interval four. It is well known that abiotic factors, including a significant increase or decrease in temperature, can cause a decrease in Fv/Fm (Leipner, 2011). Given the large range of potential Fv/Fm values for measurement interval four, the statistical model may not have reliably predicted where that actual value may lie, resulting in a perceived decrease in measurement interval five (Fig. 3).

By statistically controlling leaf temperature at a constant rate (21.76°C) across all treatments, the actual treatment effects were revealed without interference from varying temperatures (Fig. 5). When leaf temperature was held constant, a decrease in Fv/Fm in measurement interval five was still present. This indicated that the decrease was caused by treatment effects in addition to the decrease in temperature in measurement interval four. Since both control treatments (water and water+ SARC) had a decline in mean Fv/Fm in measurement interval five, as well, it may be accurate to attribute this decline to a response associated with the stress of cold temperatures in measurement interval four and the addition of a fifth foliar application, rather than on detrimental effects of the oxidant solutions alone.

ANALYSIS 2 RESULTS: CHLOROPHYLL FLUORESCENCE DATA OVER FIVE MEASUREMENT INTERVALS INCLUDING pH AND ORP MEASURES

Since pH and ORP measures were taken only when each new batch of disinfectant solutions was mixed, data were taken only five times. On the contrary, there were seven measurements for both the visual and chlorophyll fluorescence data (five measurements upon receipt and application of the new batches and two additional measurements following the final application). Due to this discrepancy pH and ORP data could only be run against visual and fluorescence data from the first five measurement intervals. Since there was no change in visual data results as a result of the presence of pH and ORP data, no new information on visual data will be presented in this analysis. The following data represents findings when pH and ORP were included as covariates in an analysis with the chlorophyll fluorescence data across five measurement intervals.

Results represent least squared means of the fluorescence data taken across five measurement intervals ($P = <0.0001$, $R^2 = 0.29$, RMSE = 0.0059) (Appendix C). The analysis resulted in the following interactions: Fv/Fm measurement interval and ORP and oxidant concentration and sarcosinate concentration, as well as the following single effects: Fv/Fm measurement interval, oxidant type, sarcosinate concentration, soil temperature, leaf temperature, and pH. Data were an average of the four replicate plants in each treatment group.

Oxidant Solution and Environmental Factors (Treatment Effects)

The control solutions had a pH of M = 7.51 and M= 7.38 for water and water + SARC, respectively. The pH of the six chlorine dioxide solutions varied very slightly, with an average of 6.6. The two hydrogen dioxide solutions had a lower pH of 4.5.

The control solutions had a mean ORP of 746 mV and 747 mV for water and water +SARC, respectively. Mean ORP of the chlorine dioxide solutions averaged 945 mV. Similarly, mean ORP of the ClO_2 + Sarc solutions were 987 mV (100 $mg \cdot L^{-1}$ ClO_2 + Sarc), 928 mV (200 $mg \cdot L^{-1}$ ClO_2 + Sarc), and 907 mV (400 $mg \cdot L^{-1}$ ClO_2 + Sarc). The hydrogen dioxide solutions had lower mean ORP of M = 665 mV and M = 633 mV for the H_2O_2 and H_2O_2 + Sarc solutions, respectively (Appendix D).

The average soil moisture of the potting media across the five measuring dates was 0.17 m^3/m^3. Across all plants and treatments, the mean soil moisture ranged from M = 0.16 m^3/m^3 at its minimum in week one to M = 0.18 m^3/m^3 at its maximum in week two.

The average soil temperature across the five measuring dates was 14.5°C. Across all plants and treatments, the mean soil temperature ranged from M = 11.2°C in week four to M = 16.0°C in week three.

The average leaf temperature across the five measuring dates was 20.6°C. Across all plants and treatments, the mean leaf temperature ranged from M = 16.7°C in week four to M = 21.7°C in week two.

Fluorescence Analysis When No Treatment Effects Are Included as Covariates in the Model

Variation in mean Fv/Fm was apparent in all treatments and during all measurement intervals as a result of soil temperature, leaf temperature, pH and ORP (Fig. 6). This variation tended to be larger in the 400 $mg \cdot L^{-1}$ ClO_2 and ClO_2 + Sarc solutions. Additionally, there tended to be more variation in measurement intervals three and four when examining trends in all five measurement intervals.

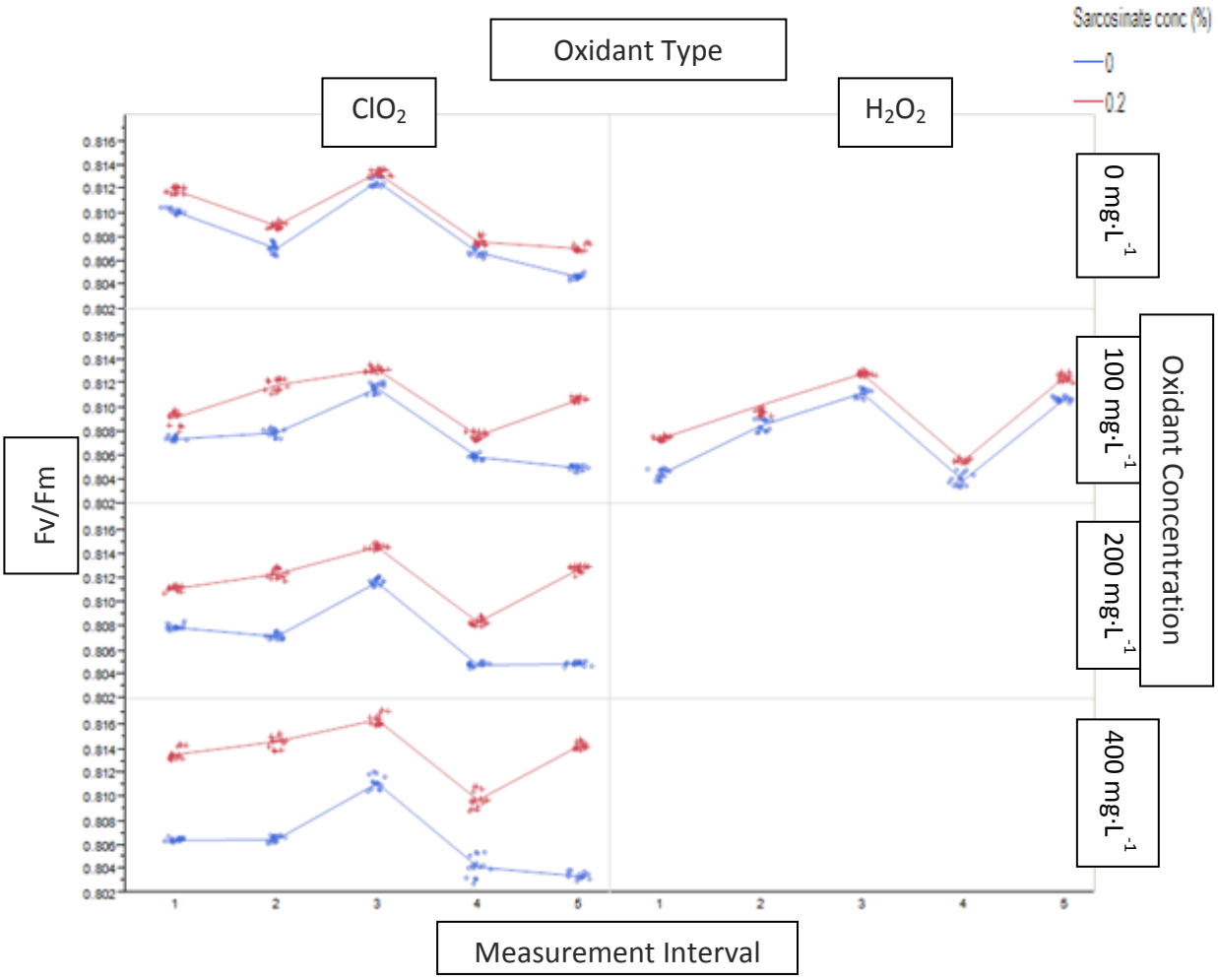

Figure 6. Point cluster variation of mean Fv/Fm due to changes in soil temperature, leaf temperature, pH, and ORP of ten oxidant treatments across five measurement intervals. Chlorine dioxide oxidant treatments are represented in the left column, while the hydrogen dioxide oxidant is represented on the right. Rows represent oxidant concentration used. Treatments with and without Sarc surfactant are present in the same quadrant, differentiated by color (None = Blue, Sarc = Red). n = 16.

Across all treatments when these variables (soil temperature, leaf temperature, pH and ORP) were not included in the model, solutions containing Sarc have higher mean Fv/Fm values (Fig. 6). These effects became more apparent as oxidant concentration increased.

Additionally, when these variables were not included in the model, there was a decrease in mean Fv/Fm values during measurement interval four in all treatments (Fig. 6). The two water controls decreased in mean Fv/Fm by similar amounts (0.006) in measurement interval four. In the water + Sarc treatment, this amount remained similar through measurement interval five. On the contrary, the mean Fv/Fm value of the water treatment continued to decrease by an additional 0.002. By measurement interval five, the ClO_2 + Sarc treatments mean Fv/Fm values had recovered to over 50% what was lost in measurement interval four. On the other hand, the ClO_2 treatments either remained at the same mean Fv/Fm value as in measurement interval four (200 $mg \cdot L^{-1}$ ClO_2), or continued to decrease in mean Fv/Fm value (100 and 400 $mg \cdot L^{-1}$ ClO_2) in measurement interval five.

Fluorescence Analysis When Treatment Effects Are Included as Covariates in the Model

This analysis was used to determine any impact treatments effect may have had on camellia chlorophyll fluorescence when treated with ClO_2 and H_2O_2. When variables were held constant, the average across all leaves, plants, and measurement intervals was used in the analysis. Those averages were: soil temperature = 14.45°C and leaf temperature = 20.57°C.

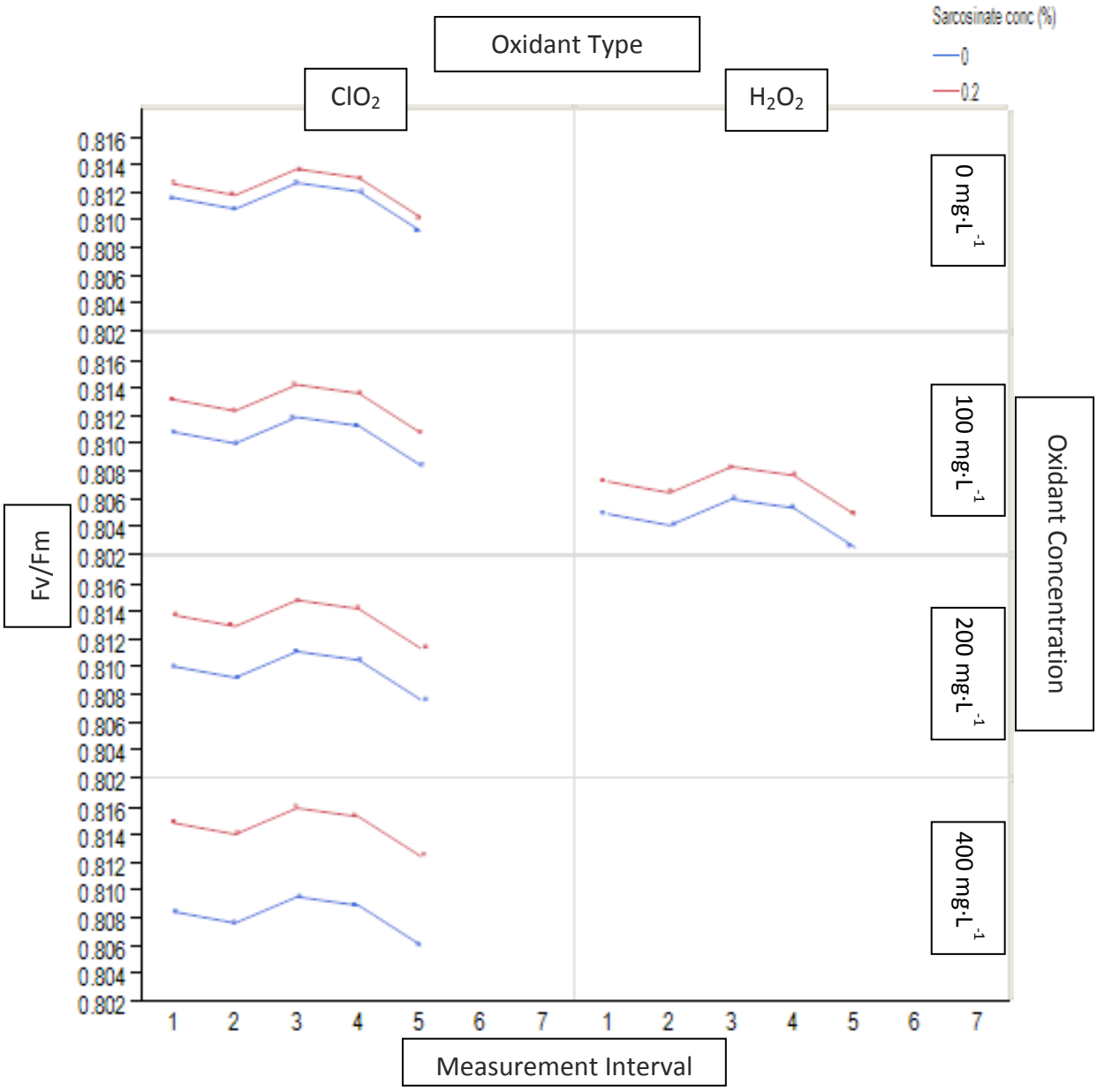

Figure 7. Mean Fv/Fm of ten oxidant treatments when one average soil temperature (14.45°C), leaf temperature (20.57°C), pH, and ORP were used across five measurement intervals. Chlorine dioxide oxidant treatments are represented in the left column, while the hydrogen dioxide oxidant is represented on the right. Rows represent oxidant concentration used. Treatments with and without Sarc surfactant are present in the same quadrant, differentiated by color (None = Blue, Sarc = Red). n = 16.

Mean Fv/Fm values of treatments containing sarcosinate were greater than mean Fv/Fm values of treatments not containing surfactant at all oxidant concentrations across the five measurement intervals (Fig. 7). This effect became more apparent with increasing oxidant concentration due to a simultaneous increase in mean Fv/Fm of treatments containing Sarc and a decrease in mean Fv/Fm of treatments without Sarc, as oxidant concentration increased.

Across all treatments, there was a decline in mean Fv/Fm during measurement interval five (Fig. 7). This decline decreased in severity slightly with increasing oxidant concentration, from a decline in mean Fv/Fm of about 0.003 (0 and 100 $mg \cdot L^{-1}$ oxidant concentration) to 0.002 (200 and 400 $mg \cdot L^{-1}$ oxidant concentration) from measurement interval four to measurement interval five.

The two hydrogen dioxide solutions had a lower mean Fv/Fm than any of the eight other solutions (Fig. 7). When compared to the 100 $mg \cdot L^{-1}$ chlorine dioxide solutions, the hydrogen dioxide solutions have a lower average mean Fv/Fm (mean Fv/Fm ClO_2 - 0.006) across the five measurement intervals.

ANALYSIS 2 DISCUSSION

Oxidant Solution and Environmental Factors

Across all treatments, leaf temperature had the greatest impact on mean Fv/Fm (Appendix B). pH, ORP, and soil temperature had a significant, but relatively minimal, impact on mean Fv/Fm (Appendix C).

In this research, as chlorine dioxide oxidant concentration increased the solution ORP decreased. When examining the ORP of 0.25, 1, and 4 mg·L chlorine solutions, mixed from a 6% sodium hypochlorite solution, the solution ORP significantly increased with increasing chlorine concentration (Lang et al., 2008). Similarly, when evaluating chlorine bleach solutions at 0, 50, 100 and 200 mg·L chlorine, solution ORP significantly increased with increasing chlorine content (Pangloli et al. 2013). The reason for the opposing results found in this research is the unique proprietary processes SRO uses to formulate their ClO_2 solutions (Peters, 2012).

Unlike other ClO_2 products on the market, SRO uses a proprietary process to mix the ClO_2 solutions, which gives Electro-BioCide its unique characteristics. This process begins by running a NaCl brine fluid though an energized electrochemical split cell (Breedlove, 2013). The cell produces two streams: 1) a reductive, alkaline NaOH solution with an ORP of about -900mV and 2) an oxidative, acidic HOCl solution with an ORP of about +1100 mV. A hypochlorite with dissolved chlorite salt ($NaClO_2$) solution is made and acidified with the HOCl solution to release the ClO_2 (Breedlove, 2013). As a result, while the HOCl solution began the process at an ORP of about +1100 (a typical 100 mg·L E-B solution), the subsequent steps use energy potential to

release ClO_2 from the $NaClO_2$, causing a decrease in ORP with increasing ClO_2 concentration (Breedlove, 2013).

Fluorescence Analysis When No Treatment Effects Are Included as Covariates in the Model

As mentioned previously, leaf temperature had the largest impact on mean Fv/Fm values. Therefore it is easy to comprehend, that a drastic decrease in mean Fv/Fm across all treatments occurred in measurement interval four when the leaf temperature dropped to 16.7°C from 21.9°C (Fig. 6). This is in agreement with Leipner (2011), who claims decreases in Fv/Fm can be a result of low temperatures.

Interestingly, it appeared that the presence of sarcosinate surfactant, in solutions containing chlorine dioxide, lead to a quicker recovery from this temperature effect (Fig. 6). One explanation for this may be that the plants remained photosynthetically healthier than plants in the ClO_2 treatments, following the first three foliar spray applications, making them more capable of dealing with the temperature stress. Alternatively, pesticide induced depressions of Fv/Fm were alleviated by the use of a brassinosteroid on cucumber, thus, Sarc may be acting in a similar way by boosting plant photosynthetic health, following the temperature stress (Xia et al., 2006).

Fluorescence Analysis When Treatment Effects Are Included as Covariates in the Model

After controlling for soil temperature and leaf temperature, to gain a better understanding of the treatment effects, mean Fv/Fm values for treatments containing Sarc remained higher than the mean Fv/Fm values for treatments not containing Sarc (Fig. 7). These

effects became increasingly true at higher oxidant concentrations, due to a simultaneous increase in mean Fv/Fm of treatments containing Sarc and a decrease in mean Fv/Fm of treatments without Sarc, as oxidant concentration increases. It appeared that chlorine dioxide solutions containing Sarc were both buffered to the increase in chemical concentration, and furthermore, have increased photosynthetic operating efficiency because of it. Since the ORP and pH of the comparable solutions (water and water + Sarc, 100 $mg \cdot L^{-1}$ ClO_2 and 100 $mg \cdot L^{-1}$ ClO_2 + Sarc , 200 $mg \cdot L^{-1}$ ClO_2 and 200 $mg \cdot L^{-1}$ ClO_2 + Sarc, and 400 $mg \cdot L^{-1}$ ClO_2 and 400 $mg \cdot L^{-1}$ ClO_2 + Sarc) remain remarkably consistent across treatments with and without surfactant, pH and ORP can be determined to have no effect in this interaction.

Interestingly, when soil temperature and leaf temperature were not included in the model, a large decrease in mean Fv/Fm occurred in measurement interval four (Fig. 6), while when soil and leaf temperature were included in the model, a large decrease in Fv/Fm occurred in measurement interval five (Fig. 7). In measurement interval five, soil temperature, leaf temperature, pH, and ORP were not different than in any other measurement interval. Therefore, it can be assumed that none of these factors caused the drastic decrease in Fv/Fm in measurement interval five. As discussed in 'Analysis 1', when the model was run without pH and ORP data, the huge variance in mean Fv/Fm due to the fluctuation of leaf temperature in measurement interval four resulted in a prediction of a large decrease in measurement interval five (Fig. 3). When leaf temperature was controlled, as it has been in this analysis, the decrease could more accurately be attributed to treatment effects (Fig. 7). Therefore, it is likely, this large decrease in mean Fv/Fm in measurement interval five was a result of both a response to

the lower leaf temperature in measurement interval four and the added stress of an additional foliar spray application.

Finally, the hydrogen dioxide solutions have a lower mean Fv/Fm across the five measurement intervals, when compared to the eight treatments containing chlorine dioxide (Fig. 7). Given the two H_2O_2 solutions had mean ORP and pH values drastically lower than any of the ClO_2 solutions (ORP M = 665 mV and M = 633 mV for H_2O_2 and H_2O_2 + SARC, respectively, and pH around 4.5), the decreased ORP and pH may be the cause of the subordinate mean Fv/Fm values. When studying the thermodynamic limitations of photosynthetic water oxidation in PSII, Zaharieva et. al. (2011) found that at lower pH (pH 3) PSII forward reactions were blocked, thus decreasing PSII functioning. Therefore, the low pH of this 100 $mg \cdot L^{-1}$ Oxidate® solution, which is being marketed for use on a variety of ornamental crops, may be contributing to lower photosynthetic functioning. When compared to the 100 $mg \cdot L^{-1}$ ClO_2 + Sarc solution, plants may have an increased mean Fv/Fm value of 0.006.

SUMMARY

This study demonstrated that 1) Electro-BioCide at a rate predicted to eradicate *Phytophthora ramorum* (200 mg·L^{-1}) should not visually damage camellia plants until after five consecutive spray applications; 2) Electro-BioCide has a positive effect on photosynthetic functioning over time; 3) The benefits of sarcosinate surfactant on mean Fv/Fm increases with increasing oxidant concentration; 4) Chlorophyll fluorescence (Fv/Fm) is able to detect stress in camellia plants up to three days earlier than visual symptoms occur; 5) Soil moisture and leaf temperature are important factors in dark-adapted maximum quantum efficiency chlorophyll fluorescence measurement and should be an important component of any research conducted using chlorophyll fluorescence measurements; and 6) When environmental factors are controlled, pH and ORP do not significantly impact visual injury or dark-adapted maximum quantum efficiency chlorophyll fluorescence of camellia plants.

These findings indicate that Electro-BioCide has the potential to be implemented as a preventative foliar treatment for defense against foliar plant pathogens, without concern for detriment to plant health. Further research should now examine the efficacy of Electro-BioCide as a disinfectant against *P. ramorum* and other foliar pathogens to determine its efficacy in eradicating pathogen infestations.

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APPENDIX A

Whole Model Test

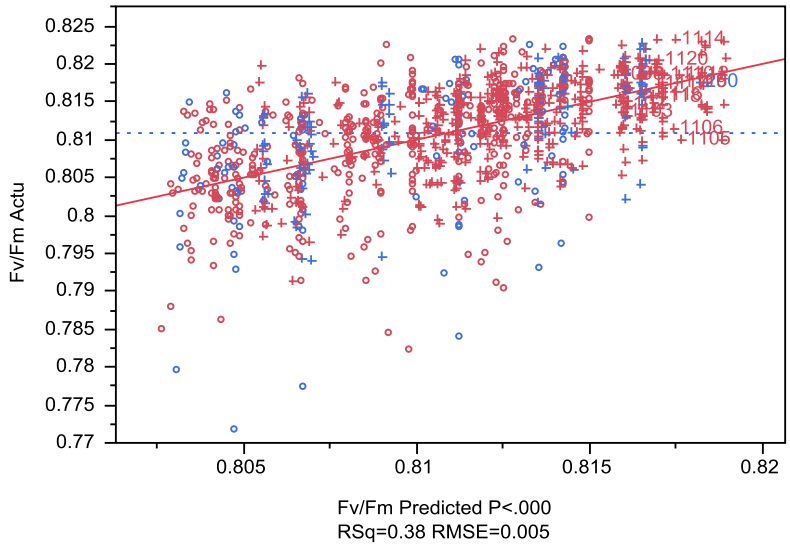
Model	-LogLikelihood	DF	ChiSquare	Prob>ChiSq
Difference	211.88527	27	423.7705	<.0001*
Full	91.87389			
Reduced	303.75916			

RSquare (U)	0.6975

Effect Likelihood Ratio Tests

Source	Nparm	DF	L-R ChiSquare	Prob> ChiSq
Oxidant	1	1	2.40671e-8	0.9999
Sarcosinate conc (%)	1	1	11.8034922	0.0006*
Fv-Fm meas. count (#)	6	6	214.418663	<.0001*
Mean(soilmstr)	1	1	1.67085004	0.1961
Mean(Oxidant conc. (ppm))	1	1	2.25235e-7	0.9996
Mean(soiltemp)	1	1	0.89837035	0.3432
Oxidant*Sarcosinate conc (%)	1	1	9.58134575	0.0020*
Oxidant*Fv-Fm meas. count (#)	6	6	18.7341687	0.0046*
Sarcosinate conc (%)*Mean(soilmstr)	1	1	5.93331381	0.0149*
Sarcosinate conc (%)*Mean(Oxidant conc. (ppm))	1	1	9.06178492	0.0026*
Fv-Fm meas. count (#)*Mean(Oxidant conc. (ppm))	6	6	36.6187062	<.0001*
Mean(Oxidant conc. (ppm))*Mean(soiltemp)	1	1	4.09505344	0.0430*

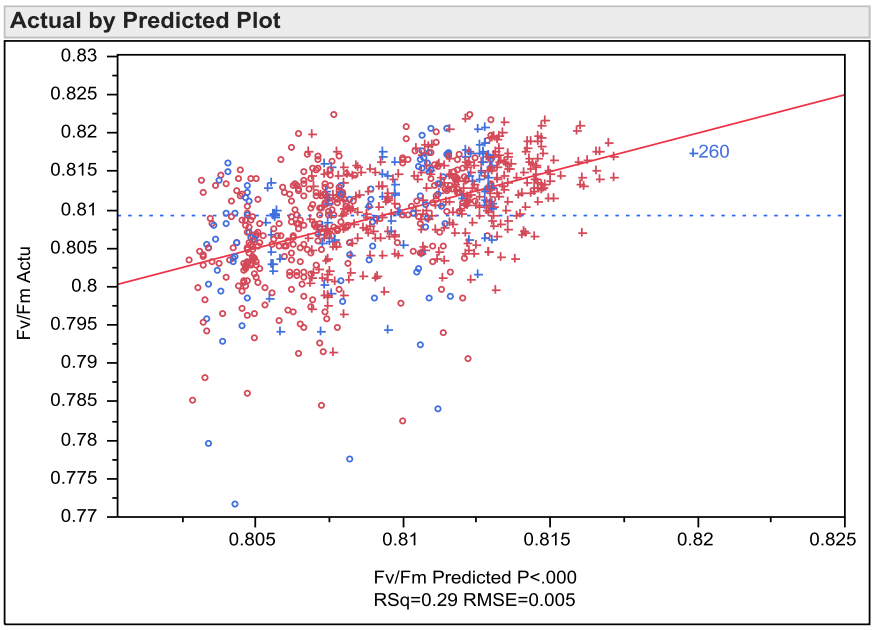
APPENDIX B



Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Fv-Fm meas. count (#)	6	6	303.7	16.7728	<.0001*
Oxidant	1	1	290	0.8985	0.3440
Oxidant conc. (ppm)	1	1	285.3	0.0037	0.9518
Sarcosinate conc (%)	1	1	280.4	54.1295	<.0001*
Tleaf	1	1	431.5	4.5572	0.0333*
Oxidant conc. (ppm)*Sarcosinate conc (%)	1	1	272.2	20.6138	<.0001*
Fv-Fm meas. count (#)*Oxidant	6	6	281.9	2.5386	0.0207*
Fv-Fm meas. count (#)*Tleaf	6	6	429.4	4.8113	<.0001*

APPENDIX C



Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Fv-Fm meas. count (#)	4	4	191.6	7.0545	<.0001*
Oxidant	1	1	185.7	17.2755	<.0001*
Oxidant conc. (ppm)	1	1	185.6	0.3776	0.5397
Sarcosinate conc (%)	1	1	192.4	47.9649	<.0001*
soiltemp	1	1	259.9	9.2517	0.0026*
Tleaf	1	1	704.6	3.8355	0.0506
pH	1	1	183.7	12.1220	0.0006*
ORP (mV)2	1	1	198	3.4984	0.0629
Fv-Fm meas. count (#)*ORP (mV)2	4	4	184.8	3.2843	0.0125*
Oxidant conc. (ppm)*Sarcosinate conc (%)	1	1	184.7	16.2829	<.0001*

APPENDIX D

	Avg ORP	**Avg pH**
Water	745.72	7.514
Water + SARC	746.98	7.38
100ppm ClO_2	997.66	6.636
200ppm ClO_2	930.42	6.554
400ppm ClO_2	906.14	6.52
100ppm H_2O_2	665.02	4.296
100ppm H2O2 + SARC	632.72	4.756
100 ppm ClO_2 + SARC	987.38	6.604
200ppm ClO_2 + SARC	928.02	6.514
400ppm ClO_2 + SARC	906.64	6.606

LIST OF ABBREVIATIONS

θPSII – Light-adapted PSII operating efficiency

ClO_2 – Chlorine dioxide

E-B – Electro-BioCide

F_m – Maximal level of fluorescence

F_o – Minimal level of fluorescence

Fv/Fm – Photosystem two maximum quantum efficiency on a dark-adapted leaf

H_2O_2 – Hydrogen dioxide

ORP – Oxidation reduction potential

PSII – Photosystem two

Q_A – Primary quinone electron acceptor of PSII

Sarc – Sarcosinate surfactant

SRO – Strategic Resource Optimization Inc.

USDA-APHIS-PPQ – United States Department of Agriculture-Animal Plant Health Inspection Service-Plant Protection and Quarantine