THESIS

THE EVALUATION OF THE POTENTIAL FOR CHLORINE DIOXIDE TO PRIME PLANT DEFENSES FOR A SYSTEMIC ACQUIRED RESISTANCE IN LIGHT RED KIDNEY BEAN PLANTS INOCULATED WITH COMMON BEAN BACTERIAL WILT

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ABSTRACT

THE EVALUATION OF THE POTENTIAL FOR CHLORINE DIOXIDE TO PRIME PLANT DEFENSES FOR A SYSTEMIC ACQUIRED RESISTANCE IN LIGHT RED KIDNEY BEAN PLANTS INOCULATED WITH COMMON BEAN BACTERIAL WILT

The induction of plant defenses is a great preventative tool for greenhouse and nursery managers to protect their plants. By priming plants with abiotic or biotic measures, managers can induce systemic acquired resistance (SAR) in plants to upregulate the ability to resist a pathogen. The accumulation of salicylic acid (SA) has been well researched and supported to be necessary for inducing SAR against pathogens. In previous research it has been shown that the functional analog of SA, acibenzolar S-methyl, has induced SAR and reduced disease severity. Acibenzolar S-methyl induces SAR when applied to plant foliage, but it does not have any antimicrobial activity to kill any pathogens on the foliage at the time of treatment. In previous research ozone has been successful at inducing SAR to reduce disease severity. Applying ozone as a treatment for greenhouse and nursery managers is not practical or safe since it is hazardous to the respiratory system. Chlorine dioxide is a powerful oxidant disinfectant that can be applied as a foliar spray to kill harmful pathogens, but it has not been reported whether it could induce plant defenses.

This research study investigated whether a commercial formulation of chlorine dioxide [Electro-biocide® (E-B)] could be used as a foliar application to plants to induce SAR. E-B is a proprietary blend of ClO_2, pH buffer, and a sarcosinate surfactant. There were a total of four spray treatments that were evaluated on plants inoculated with a bacterial wilt and on a set of

non-inoculated plants. The light red kidney bean plants were treated with E-B at 200 mg l^{-1} ClO_2, E-B 400 mg l^{-1} ClO_2, acibenzolar S-methyl (Actigard™) and a water control to evaluate disease resistance when inoculated with *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*. Treated plants were evaluated for both inoculated plants and non-inoculated plants. SA concentrations were measured five days after treatment and one day after inoculation. Leaf samples were collected to measure SA every three hours over the course of the day starting at 0700 hours and ending 2200 hours. A second SA measurement was taken at the end of the study 61 days after planting (44 days after treatment) to observe if there were any changes in SA level. Chlorophyll fluorescence measurements were taken to observe stress in response to the spray treatments and disease infection. Carbon dioxide (CO_2) gas exchange measurements were taken to observe the vigor or decline within the spray treatments and infection status. At the end of the study plants were harvested for foliage, pod, stem dry weight, and leaf area.

The first photosynthesis measurements on non-inoculated plants E-B 200 mg ClO_2 l^{-1} and 400 mg l^{-1} ClO_2 treatments declined, but recovered to control levels one week later. Inoculated plants treated with E-B and Actigard™ showed either the same or increased photosynthesis rates when compared to water. Chlorophyll fluorescence measurements indicated there was no stress due to the spray treatments. Five days after spray treatments the SA measurements showed that both concentrations of E-B resulted in an increase in SA accumulation. E-B 400 mg l^{-1} ClO_2 caused the greatest SA response. E-B 400 mg l^{-1} ClO_2 treated plant's had a 15 fold increase in SA concentrations at its highest peak when compared to water. E-B 200 mg l^{-1} ClO_2 had the second highest SA concentrations. It had a 5.9 fold increase at its highest peak when compared to water control plants. Actigard™ treated plants did not result in different SA concentrations from the water control plants. The SA concentrations levels at 44 days after treatment for all plants

that were not inoculated returned to normal levels. SA levels for inoculated plants and all spray treatments continued to rise for the duration of the study. There were no differences in biomass measurements between spray treatments. All non-inoculated plants had a greater biomass measurements when compared to all the inoculated plants.

These results conclude that E-B 200 mg l^{-1} ClO_2 and E-B 400 mg l^{-1} ClO_2 were able to prime plant defenses for SAR response. The rise in SA concentrations confirm that E-B was able to interact within the leaf as an elicitor for SAR. Unfortunately the biomass measurements for inoculated E-B treated plants did not show any difference from inoculated control plants. This indicates that the E-B treatment was not able to reduce the disease severity with CFF. Actigard (acibenzolar-S methyl) has been successful with inducing SAR and reducing disease severity in other studies. In this study Actigard was also unsuccessful in reducing disease severity. This indicates that CFF may have had too great of pressure for the inoculated plants to overcome. E-B should be investigated further with other pathogens.

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LIST OF ACRONYMS

ASM- acibenzolar-S-methyl
CFF- *Curtobacterium flaccumfaciens* pv *flaccumfaciens*
ClO_2-Chlorine dioxide
CO_2- Carbon dioxide
Conjugate SA- Conjugate salicylic acid
DAT- Days after treatment
E-B- Electro-Biocide®
EPA- Environmental Protection Agency
Fm- Maximum fluorescence
Fo- Minimum fluorescence
Fol- *Fusarium oxysporum* f. sp. *lycopersici*
Free SA – Free salicylic acid
Fv/Fm- Variable fluorescence; quantum yield for photochemistry
GST1- Glutathione S-transferase
HR- Hypersensitive response
IPM- Integrated pest management
IRGA- Infrared gas analyzer
LC-MS/MS – Liquid chromatography tandem mass spectrometry
Ml – Milliliter
Mg- Milligram
NBY- Nutrient broth yeast extract medium
Ng – Nanogram
PAL- Phenylalanine ammonia lyase
PAR- Photosynthetically active radiation
PCR- Polymerase chain reaction
PSII – Photosystem II
PR-1 – Pathogenesis related protein
PSM- *Pseudomonas syringae* pv *maculicola*
RH- Relative humidity
RNA- Ribonucleic acid
ROS- Reactive oxygen species
SA- Salicylic acid
SAR- Systemic acquired resistance
TMV- Tobacco mosaic virus
USDA-CRL- United States Department of Agriculture Crops Research Laboratory
Vaa- *Verticillium albo-atrum*
WUE – Water use efficiency

CHAPTER 1 LITERATURE REVIEW

1.1 Introduction

Plants are living organisms that are susceptible to a number of pests including insects, bacterial pathogens, viruses, and fungal pathogens. If plants succumb to a pest without intervention the results can be unsaleable plants and/or dead plants. Greenhouse and nursery managers use Integrated Pest Management (IPM) practices to produce the best plants, and to keep pests down to a minimum. IPM uses a combination of approaches to the make the best growing environment for the plant and the least hospitable environment for a pest (EPA, 2014; Managment, 2013). IPM practices identify the pests when a disease does arise and also monitors the population. IPM practices also determine what the threshold is and when to apply the appropriate pesticide (EPA, 2014; Managment, 2013). Using resistant plants is an important tool to have success against diseases (Abrol, 2013). Utilizing different tools and methods in IPM becomes more important as diseases become resistant to pesticides. In horticulture not all desirable plants are genetically resistant to diseases. Plants that are susceptible to diseases may excel in other areas that make them popular in the horticulture industry.

All plants have plant defense responses that helps to fight broad spectrum of diseases, herbivores, and abiotic stresses. Horticulturists can utilize this natural plant defense system in IPM practices. Plants can be primed for systemic acquired resistance by abiotic or biotic measures. This will ensure a faster and stronger plant defense response and the ability to be resistant to a disease. This gives the horticulturist a tool to act before a disease infects their crops and can reduce the use of pesticides (Abrol, 2013). There are spray treatments available for priming plant defenses such as acibenzolar S-methyl, which is a functional analog to salicylic

acid (Vallad and Goodman, 2004). In previous research salicylic acid accumulation has shown to be successful for inducing systemic acquired resistance in plants (Gaffney, 1993). Products such as acibenzolar S-methyl are successful at reducing disease severity, but they do not have antimicrobial activity (Neerja et al., 2013). Plants treated with the reactive oxygen species ozone have resulted in a systemic acquired response (Sharma et al., 1996). Chlorine dioxide is a powerful oxidant that disinfects surfaces on the plant, but it has not been reported if it can induce plant defenses.

This study investigated whether chlorine dioxide would be successful at inducing systemic acquired resistance in plants in order to be another tool for IPM. Light red kidney bean plants will be treated with a chlorine dioxide disinfectant or acibenzolar S-methyl while being inoculated with a vascular wilt. Inoculated plants will be stem stab inoculated with common bacterial wilt caused by the bacteria *Curtobacterium flaccumfaciens* pv *flaccumfaciens*.

There are a four hypotheses that this study tested. The first hypothesis was that there will be a SA response when plants are treated with E-B 5 days after treatment. The SA response will be much higher for E-B plants than the non-inoculated control. The second hypothesis was that the photosynthetic measurements for both non-inoculated E-B treatments will be just as efficient as the non-inoculated control. Both inoculated E-B treatments will be more efficient at photosynthesizing than the inoculated water control plants. The third hypothesis was that the chlorophyll fluorescence will not show any stress due to the E-B treatments. This will be investigated with the non-inoculated plants. The inoculated plants will show less stress when compared to inoculated control due to an induced SAR. Also, over all biocide treatments the stress levels in inoculated plants will be higher than the non-inoculated. The fourth hypothesis was that the biomass measurements will show that non-inoculated E-B will be just as good as the

non-inoculated water control. The inoculated E-B treated plants will have a higher biomass than the inoculated water control due to a reduction of disease severity. The following list are the objectives in order to test these hypotheses:

- Determine if chlorine dioxide stimulates a salicylic acid response that is crucial for systemic acquired resistance
- Determine the effects of chlorine dioxide treatments and disease on gas exchange measurements
- Measure the plant stress response to treatments and disease through chlorophyll fluorescence
- Measure the biomass of plants to determine differences between spray treatments and disease infection

1.1.1 Preliminary Research for E-B and Light Red Kidney Beans

Preliminary research to this study was done in the summer of 2012 (data not shown). The study was done by Dr. Craig Ramsey, Dr. Steven Newman, and then graduate research assistant Heather Beckmann. Light red kidney bean plants were used to investigate whether E-B induced a SAR response. Spray treatments of E-B at 200 mg ClO_2 l^{-1} were done with two different surfactants. The two different surfactants that were evaluated for this study were etaholyxloate alcohol (EA) and the other was a sarcosinate surfactant (SS). There were also a control spray treatment with tap water. All spray treatments were done with plants that were inoculated with CFF and plants that were not inoculated. The spray treatments were done 4 days before inoculation when the light red kidney beans were 9 days old. Inoculation was done by puncturing both primary leaves on each plant with a floral frog that was dipped in CFF inoculum. The two inch floral frog was metal with 30 pins that were an inch in length. The orange race of CFF was

used (B-572) and the inoculum was diluted to 10^8 colony forming units. The day of inoculation greenhouse shade cloth was drawn, exhaust fans turned off, and misters were turned on for humidity.

SA concentrations and total biomass measurements were collected at the end of the study when plants were 48-49 days old (39-40 days after treatment). Both Free and Conjugate forms of SA showed that at the end of the study the non-inoculated SA concentrations were lower than the inoculated plants. One peculiar result was that non-inoculated water control plants and inoculated water control plants had the greatest Free and Conjugate SA response. This may indicate that the non-inoculated control plants were infected with a pathogen. The non-inoculated and inoculated Free and Conjugate SA response were also not different from each other. The rise in inoculated water control plant's SA concentrations indicate that the CFF inoculation resulted in a SA response to combat the disease without treatment. The non-inoculated plants for both E-B treatments had lower SA concentration than the inoculated counter parts. Generally Free and Conjugate SA concentrations were similar for E-B SS and E-B EA for both inoculated plants and non-inoculated plants.

The total dry biomass in the preliminary study showed that both E-B EA and E-B SS that were not inoculated with CFF had a higher biomass than the water control non-inoculated. Since the non-inoculated water control plant's SA concentration indicate there may have been infection with a pathogen. This may also be the reason for the non-inoculated biomass weight being lower than both E-B treatments. The non-inoculated and inoculated water control plant's biomass weights were the same which also supports this. Both non-inoculated E-B treatments were not different from each other in biomass. For inoculated plants E-B SS had the highest biomass

weight. The water control plants had the second greatest biomass weight and E-B EA had the lowest biomass weight.

The conclusions from this study were that CFF infection elicits a SA response at the end of the study to combat the disease. Most inoculated treatments had a greater SA concentration than their non-inoculated counterparts. E-B SS had the highest biomass at the end of the study showing that it reduced the disease severity when compared to water control and E-B EA treated plants.

1.2 Common Bacterial Wilt and Beans

Curtobacterium flaccumfaciens pv. *flaccumfaciens* (CFF) is a bacterium that causes the disease common bean bacterial wilt in *Phaseolus* species (Agarkova et al., 2012). This disease was a problem for dry bean production in Colorado, Nebraska and Wyoming during the 1960's to the early 1970's (Agarkova et al., 2012; Harveson and Schwartz, 2006). The main dispersal of CFF is through infected seed, but soil and infected debris can be a reservoir of inoculum (Agarkova et al., 2012; Sammer and Reiher, 2012). Infection of CFF can result in the germinating seedlings being killed quickly or reduced seed weight (Conner et al., 2008). Seedlings that survive may be stunted and then can eventually die (Conner et al., 2008). There is no known cure for this disease. The best line of defense is to plant resistant cultivars and purchase clean seed since there is no pesticides that control CFF in common beans. (Conner et al., 2008; Yadeta and Thomma, 2013).

Common symptoms of CFF infection on *Phaseolus* are wilting of the leaves and necrotic lesions with a yellow halo on the leaves (Agarkova et al., 2012; Yadeta and Thomma, 2013). Vascular wilts are unique in that they thrive and multiply in the xylem where it is nutrient deficient (Yadeta and Thomma, 2013). The bacterium clogs up the vascular system causing a

drought-like stress that prevents the water from getting to essential parts of the plants such as the leaves (Conner et al., 2008; Yadeta and Thomma, 2013). After early 1970's CFF was not a problem for bean production in the United States until 2003. CFF remerged on dry bean production fields in Nebraska (Agarkova et al., 2012; Harveson and Schwartz, 2006). This is disease has been detrimental to dry bean producers due to CFF being on quarantine list for many countries, which prevents infected seed to be sold and imported into those countries (Agarkova et al., 2012; Conner et al., 2008). In addition to the risk of contaminated seed the disease can result in great crop losses (Agarkova et al., 2012).

1.3 Measuring Photosynthesis and Disease

Photosynthesis can be measured at the leaf level to indicate how vigorous a plant is growing. Many abiotic and biotic factors such as temperature, water status, and diseases can affect the plant photosynthesis efficiency. When stress is present it limits plant photosynthesis from reaching optimum rates. Photosynthesis is the path where plants convert water, CO_2, and light into sugar for metabolism. For instance, all plant species have an optimum temperature for photosynthetic carbon assimilation. This optimum is a hyperbolic response where anything below or above the optimum temperature, photosynthesis is reduced. Pimental et al. (2012) evaluated photosynthesis rates for two different *Phaseolus* cultivars at different temperatures. One of which was sensitive to high temperatures and the other was more tolerant to higher temperatures. Despite the different tolerances both cultivars showed an optimum photosynthesis rate at 27 ˚C (Pimentel et al., 2013).

When plants are exposed to high light levels there is extra energy not being used for photochemistry and can result in photoinhibition. Plants have mechanisms to deal with the excess energy absorbed when there is excess light. In high light conditions the electron transport

chain can be overloaded and result in the photosystem II (PSII) absorbing excess energy, which can lead to photoinhibition (Biswal, 2005). Photoinhibition creates oxidative stress due to production of reactive oxygen species (ROS) (Biswal, 2005).

Photosynthesis can decline when a plant is infected with a disease. This is dependent on how the pathogen interacts and limits the function of the plant. Resources may need to be reallocated and there may be harmful by-products from the stressor. Plants under go severe stress after an infection from a pathogen. The first sign of a pathogen is the rise in reactive oxygen species (ROS) concentrations in the plant (D'Maris Amick Dempsey 2010; Inze and Van Montagu, 2003). The result of severe oxidative stress after infection can have consequences to the electron transport chain on the thylakoid membrane, which prevents it from working properly. A study was done to evaluate plant responses to three different diseases and examine their physiological performance such as photosynthesis, transpiration, and stomatal conductance (Bassanezi et al., 2002). The diseases evaluated were monocycle of rust, angular leaf spot and anthracnose, which infect *Phaseolus vulgaris*. The results showed that as the severity of the disease increased the net photosynthesis decreased for all three diseases. Also, stomatal conductance and transpiration were generally lower.

In another study tomato plant photosynthetic responses were evaluated after inoculating two vascular wilt diseases and then compared to non-inoculated plants (Lorenzini et al., 1997). The two vascular wilt diseases used in the study were *Fusarium oxysporum* f. sp. *lycopersici* (Fol) and *Verticillium albo-atrum* (Vaa). Gas exchange measurements were done on plants 7, 14, and 21 days after inoculation (DAI). The results showed that Fol infected plants decrease in photosynthesis at 7, 14 and, 21 DAI when compared to the control. Both stomatal conductance and transpirations were lower than the control. The instantaneous water use efficiency (WUE)

was measured with the portable gas exchange system. The water use efficiency is the ratio of carbon assimilation (A) and transpiration (E) (A_{max}/E). The WUE measurements showed that the inoculated plants had a lower WUE at 7 DAI when compared to the control, but at 14 and 21 DAI the WUE for the inoculated plants surpassed that of the control.

Plants infected with Vaa at 7 DAI revealed that there was a decline in photosynthesis compared to control. Photosynthesis recovered at 14 DAI and then declined again at 21 DAI. Vaa infected plants transpiration and stomatal conductance at 7 DAI was greater than the control and then declined as the control increased. WUE for Vaa plants at 7 DAI had a response similar to the Fol plants. At 14 DAI the plants WUE surpassed the control plants, but at 21 DAI the WUE returned to the control levels. The results for Vaa and Fol infection indicated that the probable cause of the decline in photosynthesis rate to be very similar to drought stress in response to the pathogen clogging the vascular system (Lorenzini et al., 1997). Reduced stomatal conductance indicated that less water was available. Lorenzini et al. (1997) stated that by measuring asymptomatic leaves, they were able to detect the disease a week before seeing actual symptoms on the leaves.

1.4 Measuring Chlorophyll Fluorescence and Disease

When chlorophyll *a* is excited by a light photon three outcomes can happen. The photon can be used for photochemistry, dissipated as heat or it can be re-emitted as fluorescence (Jones, 2013; Maxwell and Johnson, 2000). Research has shown that chlorophyll fluorescence can be measured to investigate stress in plants. Chlorophyll fluorescence measurements start with a plant leaf that has been dark adapted to ensure no photochemistry is taking place and then flashed with a short high intensity light (Maxwell and Johnson, 2000). The Fo is the minimum fluorescence and is measured in the dark when all the PS II reaction centers are fully open. Fm is

the maximum fluorescence during a saturating light pulse and is measured when the reaction centers are closed (Jones, 2013; Maxwell and Johnson, 2000). The result of the measurement is the fluorescence being emitted by functioning reaction centers. If a plant has photoinhibition, the Fo measurement will increase indicating less PSII reaction centers available for photochemistry (Jones, 2013). The following equation can then be calculated to determine the maximum quantum yield for photochemistry : $\frac{Fm-Fo}{Fm} = \frac{Fv}{Fm}$ (Jones, 2013; Maxwell and Johnson, 2000). A Fv/Fm value that is around 0.83 is considered to be a healthy plant, but can vary from species to species (Jones, 2013). The lower the values for Fv/Fm indicate stress.

The effects of ozone were examined on chlorophyll fluorescence in a study with one year old *Tilia americana* saplings (Pellegrini, 2014). Saplings were treated with ozone for 8 hours each day for 45 days. Ozone was applied at 120 ppb. Fv/Fm measurements were taken at 8, 15, 28, 38, and 45 days while being treated. At first the ozone treated plants had similar Fv/Fm values when compared to control, which had a mean of 0.81 Fv/Fm. The ozone treated plants showed a decrease from the control at 28 days with a mean of 0.70. Researchers reported the cause of the decline in Fv/Fm was due to a raise in Fo, which indicates a rise in photoinhibition.

The previously mentioned study reported by Lorenzini et al. (1997) also investigated Fv/Fm in response to vascular wilts in tomatoes. Chlorophyll fluorescence measurements were taken 7, 14, and 21 days after inoculation. Tomato plants that were inoculated with *Fusarium oxysporum* f. sp. *lycopersici* did not show different Fv/Fm when compared to control indicating there was no decreased activity within PSII due to the disease. There was a 5% decrease in Fv/Fm at 14 DAI and was reported that it was due to a 22% decline in Fm (Lorenzini et al., 1997). Tomato plants inoculated with *Verticillium albo-atrum* did show a lower Fv/Fm when compared to control at all three measurement dates.

1.5 Systemic Acquired Resistance

Since plants are rooted in a soil and unable to move they can be susceptible to a number of diseases and pests. Plants induce a number of pathogenesis related genes that produce a broad spectrum defense against the pest when attacked (Conrath, 2006; Vallad and Goodman, 2004; Walters et al., 2005). This broad spectrum of protection is effective against a diverse group including viruses, fungi, and herbivores. This induced resistance happens after an elicitor interacts with the plant and triggers a defense response systemically throughout the plant (Conrath, 2006). The induced resistance upregulates defense genes for protection after defense signals are received. One such response is known as system acquired resistance (SAR). SAR is not a cure for most diseases, but instead is an effective way that a plant copes with the disease. The SAR response usually can result in a 20-85% decrease in disease severity (Walters et al., 2005).

An important phytohormone for inducing SAR is salicylic acid (SA) (D'Maris Amick Dempsey 2010). Chorismate can be used to synthesize SA by two different pathways. One pathway is the phenylalanine ammonia lyase (PAL) pathway and the other is through isochorismate pathway (Chen et al., 2009; D'Maris Amick Dempsey et al., 2011; Klessig and Malamy, 1994). In the PAL pathway SA can either be produced into SA through o-coumarate or benzoate. SA accumulation is necessary for SAR responses in order to upregulate pathogenesis-related genes for plant defense (Kumar, 2014; Zhang et al., 2004). Inside a plant there are Free SA or Conjugated SA forms of SA (Panina et al., 2005). Free SA is the active form of SA that is responsible for activating SAR. Conjugate SA is comprised mostly of *O*-β-*D*-glucoside within a plant and does very little to directly induce SAR in while it is in conjugate form (Panina et al., 2005). Though Conjugate SA does not directly activate a SAR, it is an important reserve to be

converted as needed to Free SA for a quick accumulation response to an infection (Panina et al., 2005).

A study evaluated the necessity of SA in SAR by transforming tobacco plants with the gene *nah-G*. When the *nah-G* gene is expressed the plant produces salicylate hydroxylase, which prevents SA from accumulating in high concentrations by converting SA into catechol (Gaffney, 1993). They transformed many different lines with *nah-G* in the plants. Tobacco plants were inoculated with tobacco mosaic virus (TMV) on the lower three leaves. After 7 days they harvested the leaf tissue for analysis of the *nah-G* gene, SA concentrations, and salicylate hydroxylase. The wild type that was inoculated with TMV showed a 185 fold increase of SA compared to non-inoculated wild type. The transformed tobacco lines resulted in varying expression of *nah-G* gene by having low, moderate and high concentrations in the mRNA of *nah-G*. One transformed line did not have any *nah-G* or salicylate hydroxylase found and had the same concentration of SA compared to wild type inoculated. The intermediate producing *nah-G* plants showed a moderate accumulation of SA. The *nah-G* transgenic plants that had high expression of *nah-G* gene showed only a two fold increase of SA. These results showed that the *nah-G* gene in the transformed plants blocks the accumulation of SA. The plants were inoculated a second time in the upper leaves to investigate the effects of the inhibited SA accumulations on SAR. The inoculated transformed plants resulted in more severe lesions on leaves than the wild type that was inoculated. There was an inverse relationship with the size of lesions and accumulation of SA. The more SA accumulated the smaller the lesions and the less SA accumulated the bigger the lesions. Gaffney et al. (1993) concluded that SA was required in order to have successful SAR.

Horticulturists can take advantage of being able to induce SAR with abiotic and biotic measures to protect their crops, which is called "priming" a plant's natural immune system (Goellner and Conrath, 2008). By treating plants with elicitors that induce SAR results in plants that are primed for a faster and stronger reaction when compared to plants that are not treated when a threat does come along (Walters et al., 2008) . Previous studies have shown the effectiveness of applying chemicals that are functional analogs to SA (Vallad and Goodman, 2004). A commercially available product in United States, called Actigard™ (Syngenta®, Basel, Switzerland), has the active ingredient acibenzolar-S-methyl (ASM) which is a functional analog to SA (Conrath, 2006; Vallad and Goodman, 2004). ASM has been proven in many studies to induce SAR and resulted in a decrease in disease severity (Vallad and Goodman, 2004; Walters et al., 2005).

A study was done with tomato plants to investigate if ASM would reduce the severity of bacterial canker disease caused by *Clavibacter michiganensis* subsp. *michiganensis* in tomato plants (Soylu et al., 2003). The tomato plants were inoculated 24, 48, 72, and 96 hours after ASM treatments. All ASM treated plants had a decrease in disease severity when comparing to the control. Though all ASM treatments resulted in decreased disease severity, the best decrease was observed when the ASM treatment was done 72 hours prior to inoculation.

Priming plants for SAR is a beneficial tool to use in an IPM plan. Determining the time interval for treatments is important in order to get SAR protection for crops. In previous studies monocots primed with ASM have a long time effect of SAR, but for dicots there was a need to do multiple applications of the ASM to reduce disease severity (Vallad and Goodman, 2004). Previous studies show the plant resource costs of priming plants may have a reduction in productivity in plant such as growth and/or crop yield (Vallad and Goodman, 2004; Walters,

2009). Heil et al. (2000) did a study to investigate the costs of priming plants with ASM when plants are not inoculated with a pathogen. They chose a fast growing wheat variety to compare ASM treated plants against control non-treated plant. There were three growth conditions under varying nitrogen concentrations. The results showed plants treated with ASM had a lower biomass and seed compared to control (Heil et al., 2000). The study may indicate why plants induce resistance only when being attacked due to the energy and resources costs that need to be reallocated from other daily metabolic needs.

In another study, rice plants were treated with ASM determine the SAR responses against *Rhizoctonia solani* (Neerja et al., 2013). The results showed a lower disease severity with inoculated ASM treated plants compared to the inoculated control. An interesting result of the study showed that there was a greater yield for non-inoculated ASM treated plants when compared to control non-inoculated plants. The costs of priming are not clear cut, but should be considered when investigating priming plants for protection.

ASM has been documented in many studies that it is successful at inducing SAR, which reduces disease severity. However, there have been reports for some plants and diseases where no reduction in disease resulted after treatment (Walters et al., 2005). Walters et al. (2005) stated, "ASM did not induce resistance to Barley yellow dwarf virus (in winter barley field trial), and under controlled conditions, ASM did not induce resistance to *Phytophthora brassicae* in *Arabidopsis* or *P. infestans* in potato". The reasoning proposed is that this may be due to genotype and environmental factors. The fitness of the genotype of the both the plant and the pathogen may affect successful SAR development as they both combat for survival.

1.6 Reactive Oxygen Species and Plant Defenses

Reactive oxygen species (ROS) are a part of everyday life inside a plant. There is a ROS balance between being functional and creating stress within the plant. ROS can become dangerous due to their affinity to take electrons. This is especially dangerous to cellular structures within a living organism where ROS can do damage (Foyer and Mullineaux, 1994). The majority of reactive oxygen species are made within the chloroplasts of a plant (Inze and Van Montagu, 2003). In chloroplasts the PSII is able to produce oxygen by splitting water. This creates an oxygen rich environment where O_2 can interact with photosynthetic processes and ROS can be created (Foyer and Mullineaux, 1994; Inze and Van Montagu, 2003). ROS can be produced by the electron transport chain. When this occurs it is called the Mehler reaction (Foyer and Mullineaux, 1994; Inze and Van Montagu, 2003). For instance, at PSI reduced ferredoxin can react with oxygen to create a superoxide when $NADP^+$ is not available (Foyer and Mullineaux, 1994). The superoxide reacts with superoxide dismutase to become hydrogen peroxide. The hydrogen peroxide can be reduced to water by ascorbate peroxidase (Foyer and Mullineaux, 1994; Inze and Van Montagu, 2003). This process helps the electron transport chain from being over reduced (Inze and Van Montagu, 2003).

A surge of ROS is one of the first reactions to when a pathogen attacks a plant (D'Maris Amick Dempsey 2010; Inze and Van Montagu, 2003). The rise in ROS can be used to directly kill the pathogen and/or become signaling molecule to activate plant defenses against the pathogen (Bartoli et al., 2013; D'Maris Amick Dempsey 2010). Salicylic acid can also be synthesized as a result from the ROS rise (D'Maris Amick Dempsey 2010). Another important role for ROS in SAR is the ability to produce a hypersensitive response (HR). HR involves an oxidative burst within a cell being attacked and then a second larger oxidative burst, which

results in cell death (Inze and Van Montagu, 2003). This cell death is believed to isolate the pathogen from spreading to the rest of the plant (Zhang et al., 2004). In susceptible plants there is either no oxidative burst or only the first small burst is present (Inze and Van Montagu, 2003).

Previous research indicated that the ROS ozone (O_3) can induce systemic acquired resistance in *Arabidopsis thaliana* (Sharma et al., 1996). Plants that were 3-4 weeks old were exposed to ozone fumigation inside chambers at 300 ppb for 6 hours. Another set was measured under ambient air. The active form of SA (Free SA) and Total SA (Conjugate SA + Free SA) were measured. Leaf tissue samples were collected for Free and Total SA concentrations during ozone treatment at 0, 3, 6, 12, and 24 hours. The results indicated that Free SA concentrations rose rapidly to their peak concentration at 3 hours, which was 3.5 to 4.5 fold higher than controls in ambient air. The Free SA then declined until back to control levels by 24 hours. Total SA concentrations continued to rise and had its peak at 24 hours where it was 3.8 to 4.7 fold higher than controls in ambient air. The study continued to determine if SA accumulation that was induced by ozone was able to induce resistance to the pathogen *Pseudomonas syringae* pv *maculicola* (PSM). Plants treated with ozone had a decrease in disease symptoms as well as reduced growth of PSM inside the plant when compared to ambient inoculated plants. Transcripts for genes that are known to be associated with resistance to a pathogen were also measured. The following ribonucleic acid (RNA) transcripts were measured: PAL glutathione S-transferase 1 (GST1) and pathogenesis related protein 1 (PR1) mRNA expression. Ozone treated plants had greater PAL, GST1 and PR1 compared to untreated controls.

1.7 Electro-Biocide®/ Chlorine dioxide

Ozone has been shown to induce SAR, but it is not an ideal candidate to use in the horticultural industry for everyday use. It is dangerous since it can be harmful to the respiratory

system of mammals and can also damage plants (Sharma et al., 1996). In order to apply the product it would have to be fumigated in the greenhouse. It is therefore not a practical nor safe application for nursery and greenhouse managers to use every day. ASM has been effective at inducing SAR in plants, but it has no antimicrobial properties (Neerja et al., 2013). Chlorine dioxide (ClO_2) is a powerful oxidant for disinfecting plant surfaces from harmful pathogens. ClO_2 can be dissolved in water while keeping its gaseous state (Kelley, 2004). ClO_2 makes an easy foliar treatment for plants since it is soluble into water without losing its oxidative properties.

Electro-biocide® (E-B) (SRO Inc., Bailey, CO) is a proprietary blend of chlorine dioxide, surfactant, and a pH buffer (SRO, 2010). The product is commonly used in the medical field to disinfect surfaces in a hospital setting. It is a powerful oxidant that is effective in killing many viruses, bacteria, and fungi (SRO, 2010). E-B could be very powerful as a pest management tool if used before the plants are attacked by a pathogen. The foliar application would serve two preventative purposes. First, the oxidative power of ClO_2 would sanitize the surfaces of the foliage before any pest gets the chance to infect the plant. The sarcosinate surfactant in E-B helps the foliar spray adhere to the leaves uniformly, and semi-plasticizes the epicuticle wax layer, which allows the product be taken up through the epidermis cuticle. The second preventative action would be the E-B activity within the plant may cause a surge of ROS, which would then induce plant defenses. The possibility of E-B interaction inside the plant could activate a SAR responses for increasing plant defenses against a wide range of pathogens.

E-B includes a pH buffer that makes the solution non-corrosive and safe. This is important for greenhouse use to prevent corrosion to equipment and greenhouse benches when plants are being treated with E-B. The Environmental Protection Agency (EPA) also gives it a

safety rating of IV (SRO, 2010). This is the safest rating that you can get from EPA meaning it is virtually non-toxic and safe to use. However, for full greenhouse and nursery registration, SRO will need additional EPA labelling for E-B to be used in ways other than a disinfectant.

CHAPTER 2 MATERIALS AND METHODS

2.1 Study Design

The greenhouse study was conducted at the United States Department of Agriculture's Crop Research Laboratory (USDA CRL) greenhouses in Fort Collins, Colorado (40°33'55.3"N 105°05'08.2"W). The study was conducted from May 22nd, 2014 until July 23rd, 2014. Once plants had germinated they were completely randomized into eight separate treatments with twelve replications in each treatment. Treatments were designated into two separate sets in order to measure them at equal distances from treatments (Table 1). This is due to there being 96 plants total and the physiological measurements completed with the portable photosynthesis system (Li-6400 XT, LICOR Lincoln, Nebraska) would have to be done on separate days.

Table 1: List of Biocide Treatments

The list of biocide spray treatments in the study. Plants were separated into two sets in order to measure physiological measurement as close as possible. Set A included both E-B treatments both inoculated and non-inoculated plants. Set B included Actigard and water treatments including inoculated and non-inoculated plants.

Biocide Type	Concentration	Inoculation Status	Set A or B
Electro-biocide®	200 ppm	Yes	Set A
Electro-biocide®	200 ppm	No	Set A
Electro-biocide®	400 ppm	Yes	Set A
Electro-biocide®	400 ppm	No	Set A
Actigard™	60 ppm	Yes	Set B
Actigard™	60 ppm	No	Set B
Water	0 ppm	Yes	Set B
Water	0 ppm	No	Set B

2.2 Plant Material

Light red kidney beans (*Phaseolus vulgaris* L.) (Johnny Select Seeds Winslow, Maine) were planted on May 22nd, 2014. They were grown in 6.8 L pot (Western Pulp, Corvallis,

Oregon). The pots were filled with Farfard's 4-MP potting medium (Sun Gro Horticulture Agawam, Massachusetts). The light red kidney beans were bush type plants, which mature in six to eight weeks. Four beans per pot were planted and then watered in to settle in the medium and ensure contact to the beans. Once the seeds germinated they were culled to the two most vigorous seedlings. Two seedlings per pot were left in order to complete two separate measurements. One seedling was used for the first salicylic acid concentration measurements and would be harvested 23 days after planting. The other seedling remained in the pot to grow for photosynthesis, fluorescence, final SA measurement and biomass measurements until the final harvest at 61-63 days after planting. Plants were fertilized with 20N-2.2P-8.3K (Jack Peters Professional Lite 20-10-20 Allentown, Pennsylvania) soluble fertilizer through fertigation methods at 100 mg $N \cdot L^{-1}$ as irrigation was needed. Plants were irrigated with clear water every 5th irrigation. After inoculation, plants were fertilized once per week. Greenhouse parameters were set for 27 ˚C day time temperatures and 17 ˚C night time temperatures.

2.3 Common Bean Bacterial Wilt Inoculation Methods

The yellow race (B-528) of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (CFF) was used to inoculate the light red kidney bean plants. On June 4, 2014 inoculation bacterium was cultured on nutrient broth yeast extract medium (NBY) and incubated at 22 ˚C. Plates were re-cultured on new NBY plates on June 9th and 10th in order to have pure cultures for June 11th and 12th. The NBY agar was prepared with the following ingredients:

- Difco^{TM} Nutrient Broth 8 g (Becton Dickinson and Company, Franklin Lakes, NJ)
- Batco^{TM} Yeast Extract 2 g (Becton Dickinson and Company, Franklin Lakes, NJ)
- K_2HPO_4 2 g

- KH_2PO_4 .5 g
- Glucose 2.5 g
- Fisher BioReagents™ Powdered Agar 15 g (Grade: Molecular Genetics, Thermo Fisher Scientific, Waltham, MA)

All ingredients were mixed in a 1000 ml of distilled water and then autoclaved for 15 minutes. Next 6.16 g of $MgSO_4$ was dissolved into 25 ml of distilled water. Then 1 ml of the solution was added to the autoclaved agar mixture once cool enough to handle with a sterile syringe and Millex®-GS 0.22 µm filter unit (Millipore Corporation Bedford, MA). Agar solution was then poured into petri dishes and allowed to continue to cool overnight under flow hood.

Greenhouse parameters were set for 32.2 ˚C and close to 100% relative humidity (RH) for inoculation one day prior to inoculation. Shade cloths were drawn and greenhouse exhaust fans were turned off. After inoculation plants were given 48 hours in the humid and hot conditions to give the best environmental conditions for a successful inoculation of CFF.

Plants were inoculated with the stem stab method at 20 days old and 4 days after spray treatment (DAT). Sterile 20 gauge BD PrescisionGlide™ (Becton Dickinson and Company, Franklin Lakes, NJ) needles were dipped in CFF pure cultures and then inserted at the cotyledon scar at a downward angle. A new sterile needle for inoculation was used for each treatment. Plants that were not inoculated were mock inoculated with sterile needles without CFF inoculum to ensure all plants were given the same mechanical and environmental treatment.

2.4 Biocide Spray Treatments

In total there were four different spray treatments. E-B was prepared by Strategic Resource Optimization, INC (Bailey, CO). Spray treatments were E-B 200 mg ClO_2 l^{-1}, E-B 400

mg ClO_2 l^{-1}, Actigard at 62 mg ASM l^{-1}, and tap water (Table 1). Set A was sprayed on June 7th, which was 16 days after planting. Set B was sprayed on June 8th, which was 17 days after planting (Figure 1). Spray treatment Set A and Set B where completed on two different days in order to have same physiological measurements days after treatment. Plants were sprayed by a low volume electrostatic sprayer (ESS Electrostatic Spraying, Watkiesville, GA). The batteries were removed since the electrostatic charge was not needed in this study. The purpose of using the ESS was because of its ability to introduce air into the water creating gentle uniform spray onto the foliage. Each plant was timed in order to get the same amount of treatment onto the foliage. Each plant was sprayed for 18 seconds of spray time. Nine seconds was given to the top of the foliage and then the underside of the foliage received nine seconds as measured with a stopwatch in order to ensure each plant received the same volume of spray.

2.5 Salicylic Acid Methods

2.5.1 Salicylic Acid Sample Collection

The first harvest for salicylic acid (SA) determination was collected 20 hours after inoculation of CFF and 5 DAT. Harvest time started at 0700 hours and ended at 1000 hours. One leaf from each plant was collected every three hours. Selection of leaves was the largest leaves for SA measurement while avoiding cotyledon leaves (unless no other leaves available). That resulted in a total of 6 harvests per plant. Plants were in humid and hot conditions throughout harvest in order to give the best inoculation environmental conditions for CFF. Final harvest for SA was collected 61 day after planting (44 days after treatment) to investigate if there were any differences in SA concentrations within spray treatments at the end of the growing season. The final harvest collection was completed in regular greenhouse conditions with temperatures 27 ˚C day time temperatures and 17 ˚C night time temperatures. At each collection

time one leaf was plucked from the plant, inserted into a sterile 50 ml centrifuge tube and quickly submerged into liquid nitrogen. Leaf samples were then placed into a -80 ˚C freezer until ready to process. Plant samples were then taken out of the -80 ˚C freezer, lyophilized for 24 hours, and then ground to a fine powder with mortar and pestle.

2.5.2 Salicylic Acid Sample Extraction

Leaf samples were analyzed at Colorado State University Analytical Toxicology Laboratory in the college of Veterinary Medicine and Biomedical Sciences. The director of the laboratory, Dr. Greg Dooley, developed a custom method for Free SA and Total SA measurement by means of liquid chromatography tandem mass spectrometry (LC- MS/MS).

Each leaf sample was measured for Free SA and Total SA following acid hydrolysis using liquid chromatography tandem mass spectrometry (LC-MS/MS). Conjugated SA was calculated by subtracting Free SA from Total SA concentrations.

Leaf samples were prepped for LC-MS/MS by measuring 15 mg (± 0.5 mg) into a 13 ml test tube. Free SA was extracted by adding 1 ml of 10% acetic acid and 20 µl of an internal standard of 15 µg/ml D4-salicylic acid. Samples were then vortexed for about 30 seconds until well mixed and then placed in a sonicating bath for 10 minutes. Samples were then vortexed for a second time for 30 seconds and placed into the sonicating bath for an addition 10 minutes. Next samples were placed into a centrifuge for 10 minutes at 3400 revolutions per minute. Making sure not to disturb the pellet, a 0.5 ml aliquot was then transferred to 1.5 ml vials for LC-MS/MS analysis of Free SA.

Total SA was extracted by acid hydrolysis. From the same sample used for Free SA, 500 µl of supernatant was transferred to a clean 13 ml glass tube and diluted with 400 µl of 10%

acetonitrile/0.1% acetic acid. Then 50 µl of 10 % hydrochloric acid solution was added and vortexed. The pH was then verified to be between 2-3 pH. Samples were then placed into an 80 ˚C sand bath for 30 minutes. Samples were then allowed to cool for about 10 minutes and then 50 µl of 15% ammonium hydroxide was added. After being vortexed samples were verified for a pH of 4 to 5. Samples were then centrifuged for 3 minutes at 14000 revolutions per minute and 0.5 ml of aliquot was transferred to 1.5 ml vial for LC-MS/MS analysis of total SA.

2.5.3 Salicylic Acid LC-MS/MS Analysis

The instrument used in the analysis was an Agilent 1290 UPLC coupled to an Agilent 6460 triple quadruple mass spectrometer, which was equipped with an electrospray ionization (ESI) source using Agilent Jet Stream Technology (Agilent, Santa Clara, CA). SA was separated on a Zorbax Eclipse Plus C18 column (2.1mm x 100mm, 3.5 µm particle size) at 40 °C (Dooley, 2015). A sample volume of 10 µL was injected and a binary mixture of 0.1% acetic acid in water (A) and 0.1% acetic acid in acetonitrile (B) at a flow rate of 0.4 mL/min. The gradient used was 20% B increasing to 100% B at 4 min, and held for 1 min. The ionization source conditions used were as follows: negative polarity, nebulizer gas flow of 5 L/min at 30 °C and 45 psi; sheath gas flow of 11 L/min at 250 °C; nozzle voltage of 500V, and the capillary voltage at 3500 V. Salicylic acid was identified by comparison of retention times with analytical standards, individual multiple reaction monitoring (MRM) mass transitions, and with MS/MS ion ratios. In this method, SA had a retention time of 2.95 minutes, the MRM transitions were 137 > 93.1 and 65.1 m/z, and an ion ratio of 9.4. Peaks matching retention within 5% with ion ratios with 20% of the standard ratio were considered acceptable for SA. D4-salicylic acid was confirmed by retention time (2.95 mins) and the MRM transition 141.1 > 69.1 m/z. The data collection and processing were performed by using Agilent MassHunter Quantitative software (v B.04.01).

2.6 Gas Exchange Measurements

Photosynthesis was conducted as a repeated measures for this study. There were two measurements conducted at 32 DAT and 39 DAT with a portable gas exchange system (LI-6400 XT, LI-COR, Lincoln, NE) (Figure 1 & 2). To ensure that plants were at the best potential to photosynthesize, the plants were well watered the morning of measurements. This ensured there was no potential for being water stressed and gas exchange could be measured easily. Plants were placed under LED lights for at least 15 minutes to acclimate to at least 1000 µmol photon m^{-2} s^{-1} PAR, which was set for the LI-6400 XT. Gas exchange was measured under the LED lights as well. This ensured that no matter what the light levels were outside the greenhouse, the plants were actively photosynthesizing at their best potential under uniform light conditions.

Three leaves for each plant were measured. The youngest, fully expanded, healthy leaves were selected to measure on each date of measurement. These leaves were prime leaves for measurement since they were actively photosynthesizing at their fullest potential. The plants that were infected with CFF began to lose leaves due to the wilt and leaves became sparse. The same selection still applied, but the availability for the prime leaves may not be available due to the disease.

Instantaneous water use efficiency (WUE) was measured with the Li-6400 XT along with other photosynthetic measurements. WUE for this study was measured at the leaf level and is calculated with the ratio of carbon assimilation (A) and transpiration (E). The WUE is calculated along with the photosynthesis measurements with the equation $\frac{A}{10^4 \times E}$. The WUE calculation was inserted to the list of computations as directed in the LI-COR 6400 XT manual (LI-COR Biosciences, 2011).

2.6.1 LI-6400 XT Settings for Photosynthesis

Photosynthetic active radiation (PAR) within the leaf cuvette was set at 1000 µmol photon m^{-2} s^{-1}. Flow rate of air for the cuvette was set at 200 µmol m^{-2} s^{-1}. Relative humidity was maintained between 50-70% RH. Block temperature was set at 20 ˚C. CO_2 mixer was set for 400 µmol mol^{-1} CO_2 concentration. After initial matching of infrared gas analyzers (IRGAs) during set up, IRGAs were matched again before each new plant (after every 3 leave measurements).

2.7 Chlorophyll Fluorescence Measurements

Chlorophyll fluorescence was conducted as a repeated measures design at 2, 7 and 25 DAT (Figure 1). Plants were dark adapted for at least 15 hours in the basement of the USDA Crop Research Laboratory greenhouses. In addition to turning the lights off they were put in an enclosed tent to ensure complete darkness. On the day of the measurement, the plants were only taken out of the tent at the time of measurement. Lights were kept off during all of the measurements. An ultra violet light was used for visual aid since it does not impact chlorophyll fluorescence measurements. The same portable gas exchange system used for photosynthesis was also used to take the chlorophyll fluorescence measurements (Li-6400 XT, LI-COR, Lincoln, NE). Three leaves were measured on each plant. At the time of chlorophyll fluorescence measurement the plants were still fairly young with few trifoliates. Two leaflets on one trifoliate was measured and a one leaflet on a different trifoliate. At each measurement date the youngest fully expanded leaves were selected as the plants continued to grow.

Soil moisture and soil temperature were also collect at chlorophyll measurements with Echo20-TM5 sensor (Decagon Devices, Inc., Pullman, WA). This instrument measures volumetric water content (m^3/m^3) within the soil and was 5 cm in length.

2.8 Biomass Measurements

Biomass was harvested at the end of the study 61-63 days after planting. Harvest time was over three days due to the amount of plant material. Biomass measurements included leaf area, leaf fresh/dry weight, stem fresh/dry weight and pod fresh/dry weight.

Leaf area was measured with a Li-3100C (LI-COR, Lincoln, NE) area meter with green and partially green leaves only. Leaves that were dead were not measured for leaf area. Above ground biomass was harvested and weighed for fresh weight. Only leaves that were attached to the plant were used for measurements. Any leaves that dropped throughout the study due to wilt were excluded from the study. This was due to the proximity of the plants making it undistinguishable from which plant it came from. After measuring fresh weight leaves, stems and pods were bagged separately and labelled according to their treatment and repetition. Bags were then put into dryer until there were no longer differences in weight.

2.9 Polymerase Chain Reaction (PCR) DNA Methods

Whatman™ FTA™ Classic cards (GE Healthcare Life Sciences Pittsburg, PA) were used to confirm the inoculation status of the light red kidney beans. All organisms have a unique genetic code that can be distinguished from other organisms. In a polymerase chain reaction (PCR) double stranded DNA is denatured by heat to become two single stranded DNA segments (Klug and Cummings, 1999). RNA primers that are complementary and unique to the DNA of interest can then be added and bind only to the specific DNA strands of CFF. This method detects the DNA of CFF to confirm or deny inoculation of the light red kidney beans for each plant in the study. Harvest for DNA was done at the end of the study when plants were being harvested at 62-63 days after planting. Fresh stems were used to extract sap that would confirm

or deny the presence of CFF. PCR cards had an indicator paper that would turn from pink to white when there was enough DNA to process. Stems were crushed with mortar and pestle to extract sap. Then the pestle was gently pressed against the indicator paper that was enclosed by a circle for each sample. Each circle was labelled to correspond with its treatment and replication. Between each sample mortar and pestle was sanitized with alcohol and was dried. DNA extraction began with non-inoculated plants followed by the inoculated plant treatments. Whatman™ FTA™ Classic cards were then dried and shipped to Dr. Leland Cseke's lab in the Department of Biological Sciences at The University of Alabama in Huntsville for PCR analysis. Dr. Cseke lab used Whatman™ FTA™ Classic Card protocol (2010) to process the cards.

2.10 Statistical Analyses

JMP 11 (SAS Cary, NC) software was used to analyze the differences in data in this study. The alpha was set at .05. All fixed effects for all analyses were limited to two way interactions. Analysis of Variance was used for measurements that were not repeated (Biomass and Last SA Harvest). Gas exchange, chlorophyll fluorescence, and first SA harvest were repeated measurements. Reduced maximum likelihood (REML) was used for repeated measurements. A random effect is used to verify there was not a problem with repeated measurements over time. The random effect used was time nesting within plant repetition. To determine differences F tests were analyzed within each interaction. These F tests were called 'Test Slices' where differences were analyzed within an interaction. To further determine differences in data there were additional F tests were performed to contrast within an interaction.

Photosynthetic measurements and chlorophyll fluorescence had CFF Status, Time and Biocide terms interacting. The models were limited to two way interactions due to the complexity of interpreting 3 way interactions. There were times where a three way interaction

with CFF status, Time, and Biocide was necessary to determine differences within the three factors, but couldn't be done due to the two way interaction limitation. The solution for this was doing three different analysis for each measurement of interest (photosynthetic or chlorophyll fluorescence). The first analysis had inoculated and non-inoculated plants analyzed together in order to determine what was happening with CFF status. The second were inoculated plants analyzed by themselves and the third was non-inoculated plants analyzed by themselves.

2014 JUNE

CALENDAR YEAR / MONTH

SUNDAY
FIRST DAY OF WEEK

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
01	02	03	04	05	06	07 Spray Set A (48 plants)
08 Spray Set B (48 plants)	09 Fv/FM Set A	10 Fv/Fm Set B	11 Inoculate Set A (24 plants)	12 Inoculate Set B (24 plants) Harvest SA Set A	13 Harvest SA Set B	14
15	16	17	18 Fv/Fm Set A (48 plants)	19 Fv/Fm Set B (48 plants)	20	21
22	23	24	25	26	27	28
29	30	01	02	03	04	05

Figure 1: Study Calendar for June

2014 JULY

CALENDAR YEAR / MONTH

SUNDAY
FIRST DAY OF WEEK

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
29	30	01	02 Fv/Fm Set A (48 plants)	03 Fv/Fm Set B (48 plants)	04	05
06	07	08 Gas Exchange Set A (24 plants)	09 Gas Exchange Set A (24 plants)	10 Gas Exchange Set B (24 plants)	11 Gas Exchange Set B (24 plants)	12
13	14	15 Gas Exchange Set A (24 plants)	16 Gas Exchange Set A (24 plants)	17 Gas Exchange Set B (24 plants)	18 Gas Exchange Set B (24 plants)	19
20	21 Harvest: Leaf Area, Fresh Weight, Last SA Harvest	22 Harvest: Leaf Area, Fresh Weight, DNA	23 Harvest: Leaf Area, Fresh Weight, DNA	24	25	26
27	28	29	30	31	01	02

Figure 2: Study Calendar for July

CHAPTER 3 RESULTS AND DISCUSSION

3.1 Salicylic Acid First Harvest

3.1.1 Free Salicylic Acid

The first harvest of Free and Conjugate SA was collected 5 DAT (1 day after inoculation). The collection process was conducted over the course of 15 hours. Statistical analysis showed CFF status had no effect on Free SA for the first harvest (Appendix I). This indicates that 22-37 hours after inoculation was too early to see the interaction of the pathogen. There were interactions between leaf tissue collection time and biocide type (Appendix I). The water control plants did not show any difference in Free SA concentrations at any collection interval (Figure 3 and Table 2). Actigard also did not show a difference in concentration of Free SA from 0700 to 2200 hours. Actigard and water were not different from each other at all time collections.

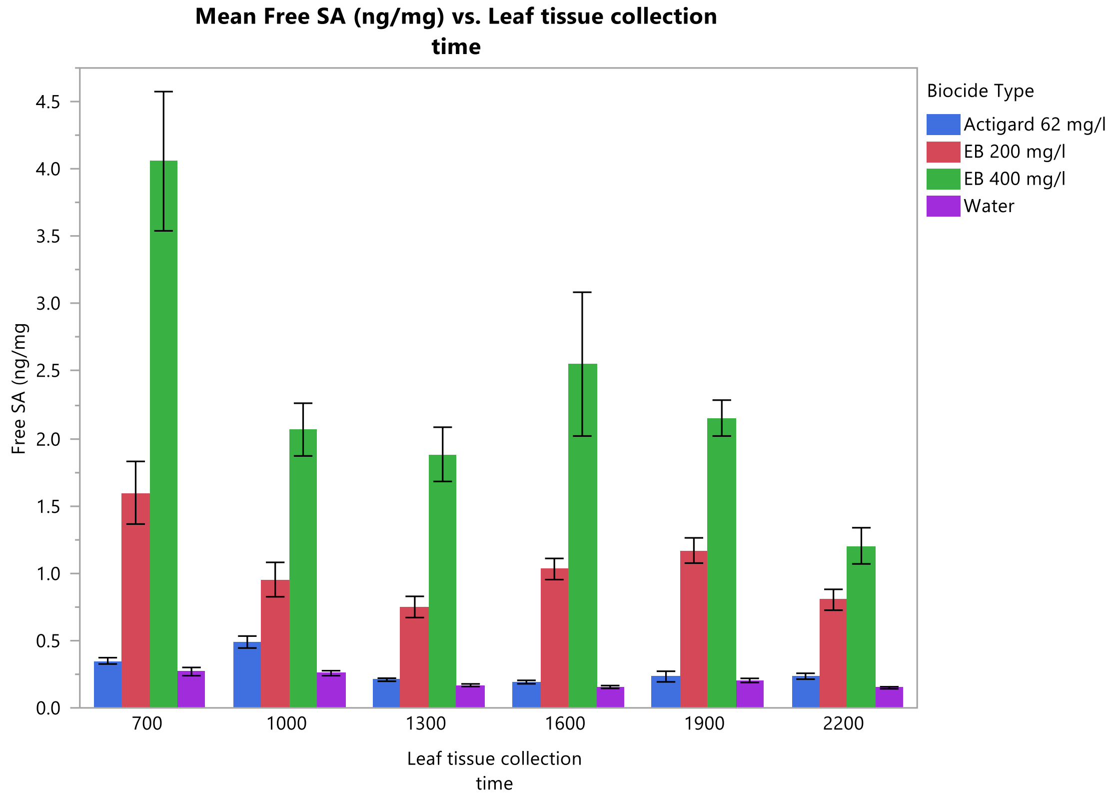

Figure 3: Mean Free Salicylic Acid versus Leaf Collection Time

Mean Free SA (ng/mg) at leaf collection in military time from 0700 to 2200 hours which totals six collection times. Actigard is represented by blue, E-B 200 mg ClO_2 l^{-1} represented by the red, E-B 400 mg ClO_2 l^{-1} represented by the green and water represented by purple. Standard error is represented by black error bars (n=24).

Table 2: First Harvest for Free Salicylic Acid

Student T Test with an alpha of .05. The T test was done from the interaction of leaf collection time and biocide type. The level is ordered by leaf collection time (military time) and then followed by biocide treatment (n=24). On the right side of the table is the least squares means of Free Salicylic (ng/mg). All levels that are not attached by the same letter are significantly different.

Level		**Least Squares Mean**
700,Actigard 62 mg/l	H I J	0.3491667
700,E-B 200 mg/l	D E	1.5970833
700,E-B 400 mg/l	A	4.0582655
700,Water	I J	0.2695833
1000,Actigard 62 mg/l	G H I J	0.4887500
1000,E-B 200 mg/l	F G	0.9525000
1000,E-B 400 mg/l	B C D	2.0658333
1000,Water	J	0.2575000
1300,Actigard 62 mg/l	J	0.2091667
1300,E-B 200 mg/l	F G H I	0.7491667
1300,E-B 400 mg/l	C D	1.8817345
1300,Water	J	0.1687003
1600,Actigard 62 mg/l	J	0.1916667
1600,E-B 200 mg/l	F	1.0308333
1600,E-B 400 mg/l	B	2.5516667
1600,Water	J	0.1556568
1900,Actigard 62 mg/l	J	0.2325000
1900,E-B 200 mg/l	E F	1.1683333
1900,E-B 400 mg/l	B C	2.1512500
1900,Water	J	0.2037500
2200,Actigard 62 mg/l	J	0.2350000
2200,E-B 200 mg/l	F G H	0.8025000
2200,E-B 400 mg/l	E F	1.2029167
2200,Water	J	0.1491667

Leaf tissue treated with E-B 400 mg ClO_2 l^{-1} had a greater concentration of Free SA compared to all other spray treatments (Figure 3 and Table 2). Plants treated with both concentrations of E-B spray treatments had an increase of Free SA. E-B 400 mg ClO_2 l^{-1} had the greatest level of Free SA at 0700 hours. It was a 15 fold greater than the water control at 0700 hours. The leaf tissue collected at 1000 through 1900 hours had similar levels of Free SA, but lower than what was measured at 0700 (Table 2). The lowest Free SA concentration for the E-B

400 mg ClO_2 l^{-1} treated plants was at 2200 hours, but was still an 8 fold greater when compared to the water control at 2200 hours. E-B 400 mg ClO_2 l^{-1} was consistently higher than Actigard and water treated plants from 0700 to 2200 hours. At 2200 hours E-B 400 mg ClO_2 l^{-1} was not different from E-B 200 mg ClO_2 l^{-1} treated plants, but was much greater in concentration for other time collections. Leaf tissue treated with E-B 200 mg ClO_2 l^{-1} had the second greatest concentration of Free SA. Leaf tissue treated with E-B 200 mg ClO_2 l^{-1} and collected at 0700 hours was 2.5 fold lower in concentration of Free SA than tissue treated with E-B 400 mg ClO_2 l^{-1}, but it was 5.9 fold greater than the water control. When compared to E-B 400 mg ClO_2 l^{-1}, generally E-B 200 mg ClO_2 l^{-1} had Free SA concentrations that were 2.2- 2.5 fold decrease from 0700 to 1600 hours. This may indicate that the doubling in concentration from 200 mg ClO_2 l^{-1} to 400 mg ClO_2 l^{-1} is highly correlated to the increase of Free SA response. E-B 200 mg ClO_2 l^{-1} was higher than water treated plants for all collections times.

3.1.2 Conjugate Salicylic Acid

The first harvest for Conjugate SA showed interactions between leaf tissue collection time and CFF wilt status; and leaf tissue collection time and biocide type (Appendix I). The interaction with leaf tissue collection time and biocide showed that at each collection time there were differences between the biocides (Figure 4 and Table 3). CFF wilt status was not significant as a major effect. When all the biocides are combined the inoculated plants were lower in Conjugate SA concentrations than non-inoculated plants at 0700 hours and 1600 hours. However, there was no interaction with CFF wilt status and biocide type. For each individual biocide there were no differences between inoculated and non-inoculated plants in their Conjugate SA concentrations. Since there were no differences for each biocides whether they were inoculated or not indicates that it was too early to detect the CFF influence.

Actigard and water were not different from each other for all leaf tissue collection times whether they were inoculated or not (Figure 4 and Table 3). The lack of Free and Conjugate SA concentrations for Actigard is likely due to ASM being a functional analog of SA. It doesn't necessarily induce a SA response without the recognition of a pathogen. E-B on the other hand does induce an SA response due to the ROS interaction within the plant (Sharma et al., 1996). Since the first harvest for SA was too early for interaction with the CFF there was not a response with water control or Actigard treated plants. If the interaction of CFF would have been present there may have been some differences with the water control.

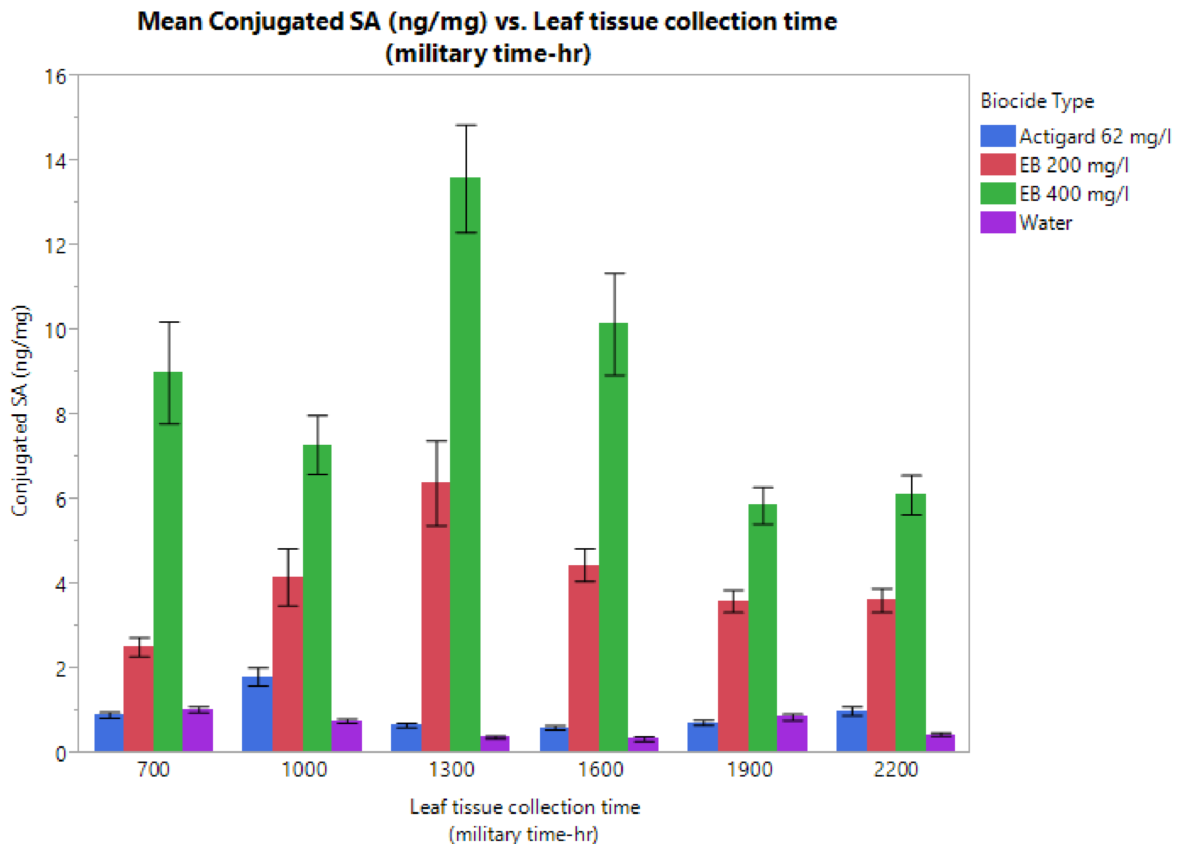

Figure 4: Mean Conjugate Salicylic Acid versus Leaf Collection Time

Mean Conjugate SA (ng/mg) at leaf collection in military time from 0700 to 2200 hours which is a total of six collection times. Actigard is represented by blue, E-B 200 mg ClO_2 l^{-1} represented by the red, E-B 400 mg ClO_2 l^{-1} represented by the green and water represented by purple. Standard error is represented by black error bars (n=24).

Table 3: First Harvest for Conjugated Salicylic Acid

Student T Test with an alpha of .05. The T test was done from the interaction of leaf collection time and biocide type. The level is ordered by leaf collection time (military time) and then followed by Biocide treatment (n=24). On the right side of the table is the least squares means of Conjugate SA (ng/mg). All levels that are not attached by the same letter are significantly different.

Level									Least Squares Mean
700,Actigard 62 mg/l								H	0.912917
700,E-B 200 mg/l						F	G		2.520417
700,E-B 400 mg/l		B							8.963988
700,Water							G	H	1.046667
1000,Actigard 62 mg/l							G	H	1.816250
1000,E-B 200 mg/l					E				4.172083
1000,E-B 400 mg/l			C						7.296250
1000,Water								H	0.775417
1300,Actigard 62 mg/l								H	0.668333
1300,E-B 200 mg/l			C						6.391250
1300,E-B 400 mg/l	A								13.574436
1300,Water								H	0.406282
1600,Actigard 62 mg/l								H	0.617500
1600,E-B 200 mg/l				D	E				4.459167
1600,E-B 400 mg/l		B							10.153333
1600,Water								H	0.321555
1900,Actigard 62 mg/l								H	0.735000
1900,E-B 200 mg/l					E	F			3.602917
1900,E-B 400 mg/l			C	D					5.861250
1900,Water								H	0.865417
2200,Actigard 62 mg/l								H	1.008750
2200,E-B 200 mg/l					E	F			3.626250
2200,E-B 400 mg/l			C						6.113333
2200,Water								H	0.459167

Both E-B treatments had the greatest concentration of Conjugate SA (Figure 4 and Table 3). Conjugate SA showed E-B 400 mg ClO_2 l^{-1} having the highest Conjugate SA and E-B 200 mg ClO_2 l^{-1} having the second highest. E-B 400 mg ClO_2 l^{-1} had its highest peak of Conjugate SA at 1300 hours. The difference in water and E-B 400 mg ClO_2 l^{-1} at this peak was a 33.1 fold increase for E-B 400 mg ClO_2 l^{-1}. E-B 200 mg ClO_2 l^{-1} had the second highest concentration and

had its peak at 1300 hours. This was a 15.6 fold increase for E-B 200 mg ClO_2 l^{-1} at 1300 when compared to water control.

The Conjugate SA was much higher in E-B treatments compared to water. This shows that the plants were still actively producing SA 5 DAT and the Free SA was not converted from a previous Conjugate SA reservoir. Previous studies have shown that SA accumulation is necessary to acquire SAR and produce parthenogenesis-related proteins (D'Maris Amick Dempsey 2010, Kumar, 2014, Zhang et al., 2004, Gaffney 1993). The SA accumulation indicate that the plants were successfully primed for SAR prior to inoculation for E-B 200 mg ClO_2 l^{-1} and E-B 400 mg ClO_2 l^{-1}. Plants treated with the oxidant ozone showed the same increase in SA accumulation and showed important defense genes were being produced (Sharma et al., 1996).

3.2 Salicylic Acid Second Harvest

The second harvest for SA analysis was taken at the end of the study at 61 days after planting (44 DAT). There was one collection per plant. Non-inoculated E-B 400 mg ClO_2 l^{-1} and E-B 200 mg ClO_2 l^{-1} had a SA response at the first harvest, but the last harvest shows that SA levels went back down to the same concentration as the control. The only difference in Free SA concentrations were between inoculated and non-inoculated plants (Figure 5 and Table 4). All non-inoculated Actigard, E-B 200 mg ClO_2 l^{-1}, E-B 400 mg ClO_2 l^{-1} and water treatments had no difference in Free and Conjugate SA for the last harvest (Figure 5 & 6). This agrees with other studies indicating dicot plants may need to have multiple spray treatments to have protection. Future studies can research when the Free SA concentrations for E-B treatment go down to after application of ClO_2 and when to reapply.

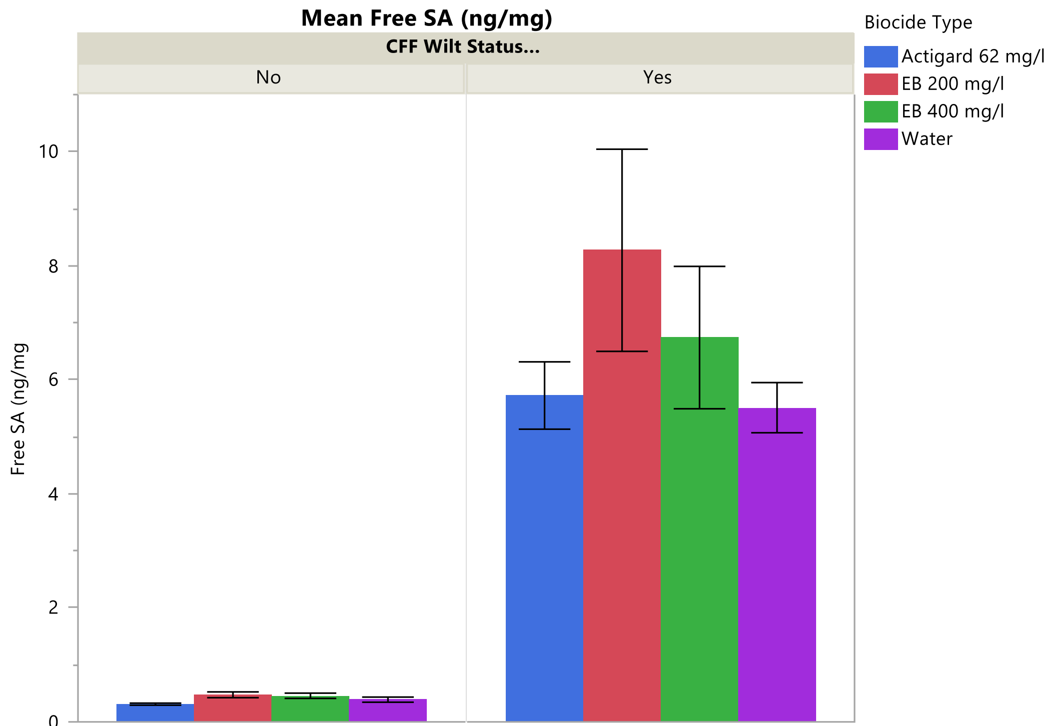

Figure 5: Mean Free Salicylic Acid for Second Harvest

Mean Free SA (ng/mg) for one collection time at the end of the study (61 days after planting). The graph is separated by CFF status with non-inoculated on the left and inoculated plants on the right. Actigard is represented by blue, E-B 200 mg ClO_2 l^{-1} represented by the red, E-B 400 mg ClO_2 l^{-1} represented by the green and water represented by purple. Standard error is represented by black error bars (n=12).

Table 4: Second Harvest for Free Salicylic Acid

Student T Test with an alpha of .05. The T test is done for CFF status. The level is ordered by inoculation status (yes or no) (n=12). CFF status was the only difference for Free SA on the second harvest. On the right side of the table is the least squares means of Free SA (ng/mg) for second harvest. All levels that are not attached by the same letter are significantly different.

Level			Least Sq Mean
Yes	A		6.5606250
No		B	0.4085417

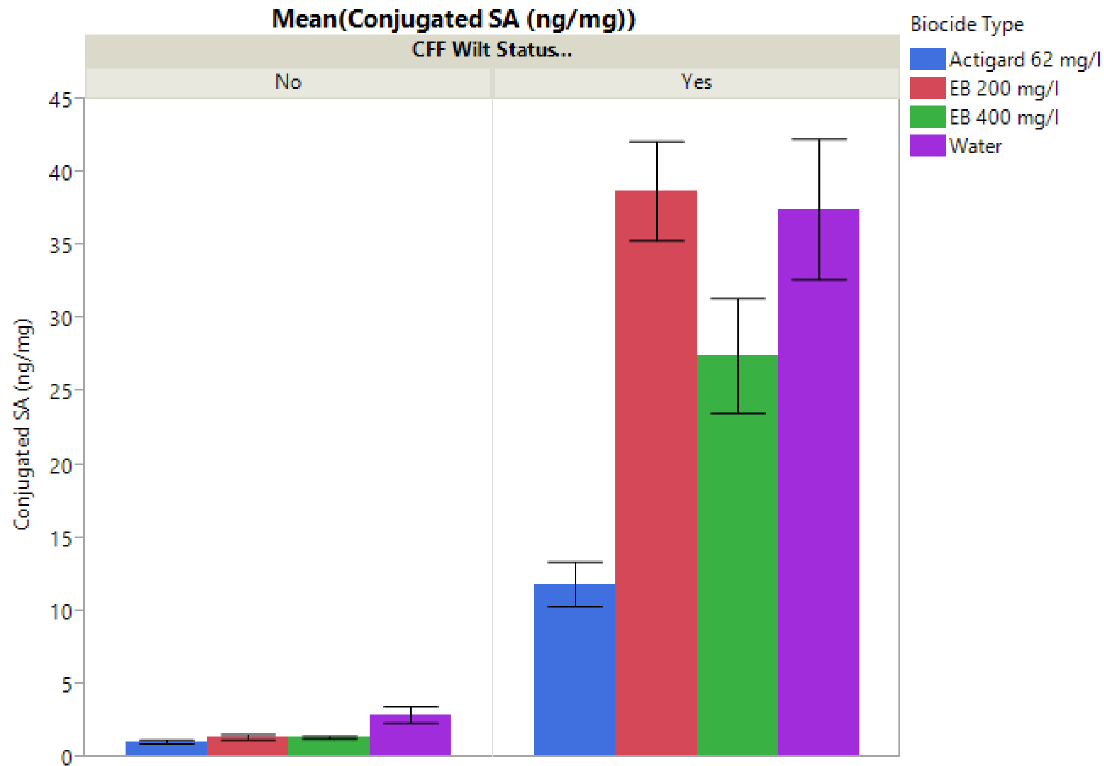

Figure 6: Mean Conjugate Salicylic Acid for Second Harvest

Mean Conjugate SA (ng/mg) for one collection time at the end of the study (61 days after planting). The graph is separated by CFF status with non-inoculated on the left and inoculated plants on the right. Actigard is represented by blue, E-B 200 mg ClO_2 l^{-1} represented by the red, E-B 400 mg ClO_2 l^{-1} represented by the green and water represented by purple. Standard error is represented by black error bars (n=12).

Table 5: Second Harvest for Conjugate Salicylic Acid

Student T Test with an alpha of .05. The T test was done from the interaction of CFF status and biocide type. The level is ordered by inoculation status (yes or no) and then followed by Biocide treatment (n=12). On the right side of the table is the least squares means for Conjugate SA (ng/mg) for second harvest. All levels that are not attached by the same letter are significantly different.

Level		Least Squares Mean
Yes, E-B 200 mg/l	A	38.748333
Yes, Water	A	37.505000
Yes, E-B 400 mg/l	B	27.486667
Yes, Actigard 62 mg/l	C	11.862500
No, Water	D	2.919167
No, E-B 200 mg/l	D	1.396667
No, E-B 400 mg/l	D	1.365000
No, Actigard 62 mg/l	D	1.060833

The SA response for inoculated plants continued to climb in all biocide treatments when compared to the first harvest. Inoculated Actigard and control treated plants at the first harvest did not show any responses for the first harvest due to being too early for wilt interaction (Figure 3 & 4). At the last harvest both Actigard and Control inoculated plants rose in SA levels for both Free and Conjugate SA (Table 4 and 5).

Inoculated E-B 200 mg ClO_2 l^{-1} and water control plants had the highest mean concentrations for Conjugate SA and were not different from each other (Figure 6 and Table 5). The second highest in Conjugate SA was with E-B 400 mg ClO_2 l^{-1} treated plants. Inoculated Actigard had the lowest of all spray treatments. The high concentrations indicate the plants were still fighting hard to combat CFF.

3.3 Salicylic Acid Conclusions

The first harvest for SA measurements showed that both E-B treatments had an increase in both Free and Conjugate SA. This agrees with the hypothesis made that E-B would activate a

SA response. These results show that when E-B is sprayed onto foliage it is able to be taken in and interact within leaves to elicit a SA response. SA is crucial for inducing a SAR response to protect plants from harmful pathogens. The first harvest was too early to see the CFF influence one day after inoculation for Free and Conjugate SA. This concludes that the Free and Conjugate responses were due to the E-B treatment given to the plants. The spray treatment was 5 days before leaf tissue was harvested for SA measurements. The results showed plants treated with E-B were still actively producing SA. The water control plants confirmed this result since the Free and Conjugate SA concentrations were nominal and never changed in concentration. These results show that E-B elicits a SA response for protection for at least 5 days after spraying and it could help fight possible infections in the horticulture industry. Also, E-B has a two part defense system to protect plants. First is the oxidizing power of chlorine dioxide to kill pathogens on the surface of the plants foliage, but also elicit a SA response that will heighten the plant defenses from future infections.

The last harvest that was done at the end of the study 44 DAT (62 days after planting) showed that all non-inoculated plants had nominal SA concentrations for both Free and Conjugate SA. The preliminary study prior to this one had a non-inoculated control with increased SA concentrations at the end of the study. This result confirms that the non-inoculated control in the preliminary study should have not been as high as it was and was more than likely due to a pathogen infection which would account for its low biomass weight. Since the non-inoculated treatments for this study all were down to normal levels of SA concentration this indicates that E-B would need multiple sprays in order to have protection over long periods of time. At 5 days after treatment E-B plants were still producing SA for protection. Future studies will need to find out at what time does the SA response stop and what time interval would be

best for spray treatments for constant protection. The inoculated plants all showed an increase in both Free and Conjugate SA. Preliminary research showed inoculated control plants had a rise in SA and this study also confirms that plants have a rise in SA to combat CFF infection.

3.4 Photosynthesis Measurements

There were repeated measurements for photosynthetic measurements (photosynthesis rates, instantaneous water use efficiency, transpiration, and stomatal conductance). These measurements were taken 32 and 39 DAT. The inoculated and non-inoculated plants were analyzed together to get an understanding of what happened with photosynthesis rates between the two. The fit model was limited to two way interactions due to the complexity of analyzing three way interactions. This means analyzing biocide treatment, time, and CFF status could not be analyzed at once. The data was first analyzed with both inoculated and non-inoculated plants to get an understanding of what was happening between the two. The inoculated and non-inoculated were then analyzed again separately to obtain the differences within biocides and days after treatment for all photosynthetic measurements and chlorophyll fluorescence.

When inoculated and non-inoculated plants were analyzed together there were interactions with biocide type and CFF inoculation status, and with DAT and biocide type. The results showed that generally the non-inoculated plants had higher photosynthesis rates (mol CO_2 m^{-2} s^{-1}) than the non-inoculated when combined both measurement dates (Figure 7 and Table 6). The only exception was E-B 400 mg l^{-1} ClO_2, which did not have a difference in photosynthesis rates between inoculated and non-inoculated plants. The water control and Actigard treatments showed a general decline in photosynthesis over time (inoculated and non-inoculated combined) (Table 7). E-B 200 mg l^{-1} ClO_2 and E-B 400 mg l^{-1} ClO_2 stayed the same from 32 to 39 DAT (Table 7).

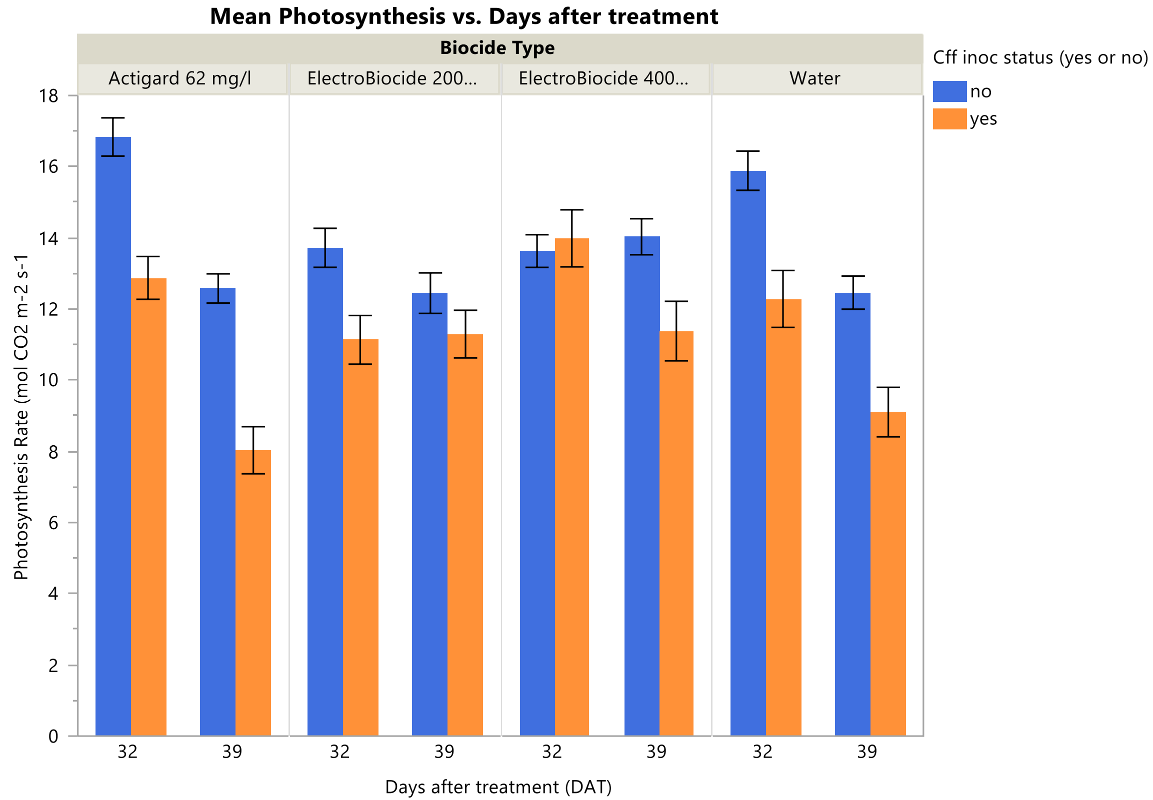

Figure 7: Mean Photosynthesis versus Days after Treatment

Mean photosynthesis assimilation (µmol CO_2 m^{-1} s^{-1}) on the y axis versus days after treatment along the x axis. The graph is separated by biocide treatment Actigard, E-B 200 mg ClO_2 l^{-1}, E-B 400 mg ClO_2 l^{-1} and water. Inoculated plants are represented in orange and non-inoculated plants are represented in blue. Standard error is represented by black error bars (n=12).

Table 6: Photosynthesis: Biocide Type and CFF Inoculation Interaction

Student T Test with an alpha of .05. The T test was done from the interaction of CFF status and biocide type. The level is ordered by biocide treatment and then followed by CFF status (yes for inoculated or no non-inoculated) (n=12). On the right side of the table is the least squares means for photosynthesis rates (µmol CO_2 m^{-1} s^{-1}). All levels that are not attached by the same letter are significantly different.

Level					Least Squares Mean
Actigard 62 mg/l, no	A				14.707063
Water, no	A	B			14.173953
Electro-Biocide 400 mg/l, no	A	B	C		13.830455
Electro-Biocide 200 mg/l, no		B	C		13.083282
Electro-Biocide 400 mg/l, yes			C		12.684161
Electro-Biocide 200 mg/l, yes				D	11.215135
Water, yes				D	10.694133
Actigard 62 mg/l, yes				D	10.453664

Table 7: Photosynthesis: Days after Treatment and Biocide Type Interaction

Student T Test with an alpha of .05. The T test was done from the interaction of days after treatment (DAT) and biocide type. The level is ordered by DAT and then followed by biocide type (n=12). On the right side of the table is the least squares means for photosynthesis rates (µmol CO_2 m^{-1} s^{-1}). All levels that are not attached by the same letter are significantly different.

Level						Least Squares Mean
32,Actigard 62 mg/l	A					14.854716
32,Electro-Biocide 200 mg/l			C			12.427030
32,Electro-Biocide 400 mg/l	A	B				13.808314
32,Water	A					14.085261
39,Actigard 62 mg/l					E	10.306010
39,Electro-Biocide 200 mg/l			C	D		11.871387
39,Electro-Biocide 400 mg/l		B	C			12.706303
39,Water				D	E	10.782825

3.4.1 Photosynthesis Non-inoculated Plants

At 32 DAT photosynthesis rates for plants that were not inoculated with CFF showed E-B 200 mg ClO_2 l^{-1} and E-B 400 mg ClO_2 l^{-1} had a decline below control and Actigard (Figure 8 and Table 8). E-B 200 mg ClO_2 l^{-1} and E-B 400 mg ClO_2 l^{-1} were not different from each other. When compared to water control E-B 200 and E-B 400 mg ClO_2 l^{-1} had about 14% decrease.

Plants treated with Actigard and water were not different from each other at 32 DAT. At 39 DAT Actigard, E-B 200 mg ClO_2 l^{-1}, and water treatments for non-inoculated plants had photosynthetic rates that were not different (Table 8). Non-inoculated E-B 400 mg ClO_2 l^{-1} had the greater photosynthesis rate at 39 DAT than non-inoculated water control and E-B 200 mg ClO_2 l^{-1} treatments. This indicates that the decline that E-B 200 mg ClO_2 l^{-1} and E-B 400 mg ClO_2 l^{-1} had at 32 DAT had fully recovered by 39 DAT. The photosynthesis rates for Actigard and water declined from 32 DAT to 39 DAT (Figure 8). The decline to non-inoculated water control was probably due to the short life span of this bush type kidney beans, which spanned approximately two months. Previous research has shown that photosynthesis declines as plants mature and enter senescence. E-B 200 mg ClO_2 l^{-1} and E-B 400 mg ClO_2 l^{-1} did not decline from 32 to 39 DAT.

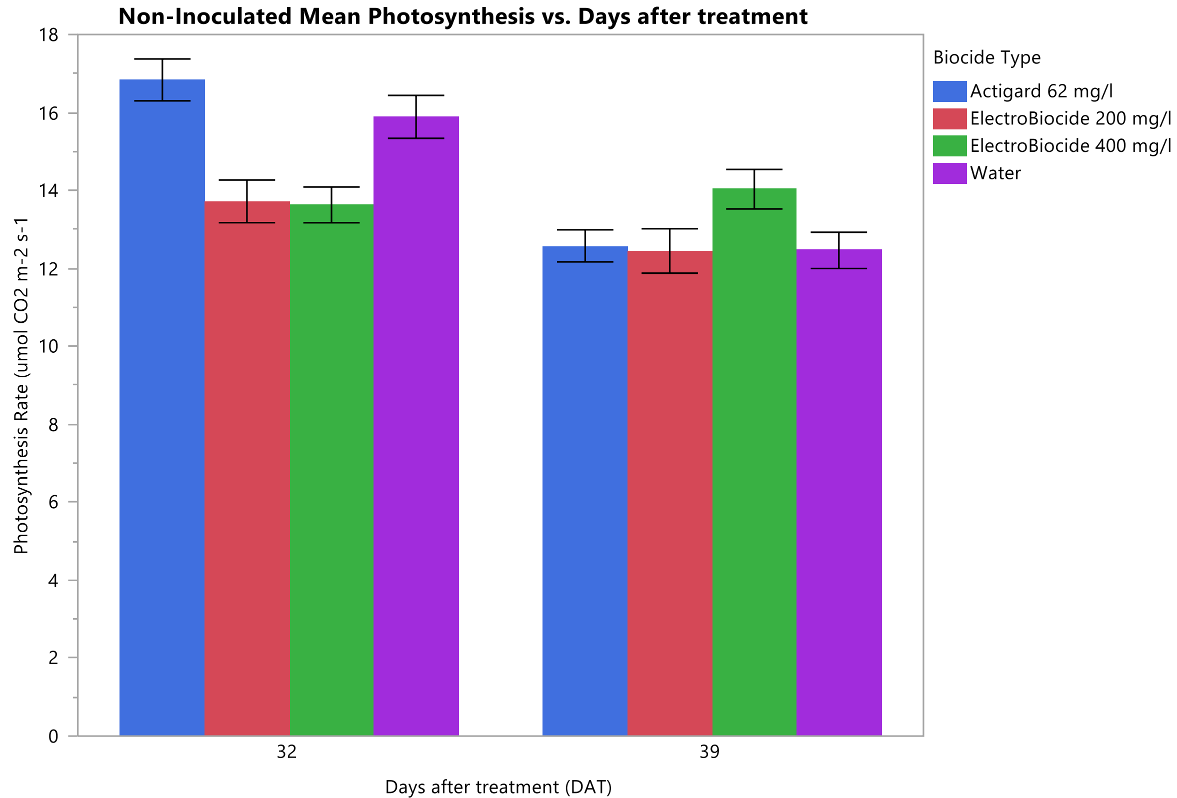

Figure 8: Non-inoculated Mean Photosynthesis versus Days after Treatment

Mean photosynthesis assimilation (μmol CO_2 m^{-1} $s^{-1)}$ for non-inoculated plants on the y axis versus days after treatment along the x axis. Actigard is represented by blue, E-B 200 mg ClO_2 l^{-1} represented by the red, E-B 400 mg ClO_2 l^{-1} represented by the green and water represented by purple. Standard error is represented by black error bars (n=12).

Table 8. Non-inoculated Plant Photosynthesis

Student T Test with an alpha of .05. The T test was done from the interaction of days after treatment (DAT) and biocide type. The level is ordered by DAT and then followed by Biocide treatment (n=12). On the right side of the table is the least squares means for non-inoculated photosynthesis rates (μmol CO_2 m^{-1} s^{-1}). All levels that are not attached by the same letter are significantly different.

Level				Least Squares Mean
32,Actigard 62 mg/l	A			16.835511
32,Electro-Biocide 200 mg/l		B	C	13.719935
32,Electro-Biocide 400 mg/l		B	C	13.629386
32,Water	A			15.887143
39,Actigard 62 mg/l		B	C	12.578614
39,Electro-Biocide 200 mg/l			C	12.446629
39,Electro-Biocide 400 mg/l		B		14.031524
39,Water			C	12.460762

3.4.2 Photosynthesis Inoculated Plants

E-B 200 mg ClO_2 l^{-1} inoculated plants at 32 DAT resulted in photosynthesis rates that were lower than E-B 400 mg ClO_2 l^{-1} (Figure 9 and Table 9). E-B 200 mg l^{-1} ClO_2 was not different from Actigard and water control plants. Actigard, E-B 400 mg ClO_2 l^{-1} and water did not show any differences from each other in photosynthetic rates. When comparing E-B 200 mg ClO_2 l^{-1} mean photosynthesis to E-B 400 mg ClO_2 l^{-1} there was a 21% decrease.

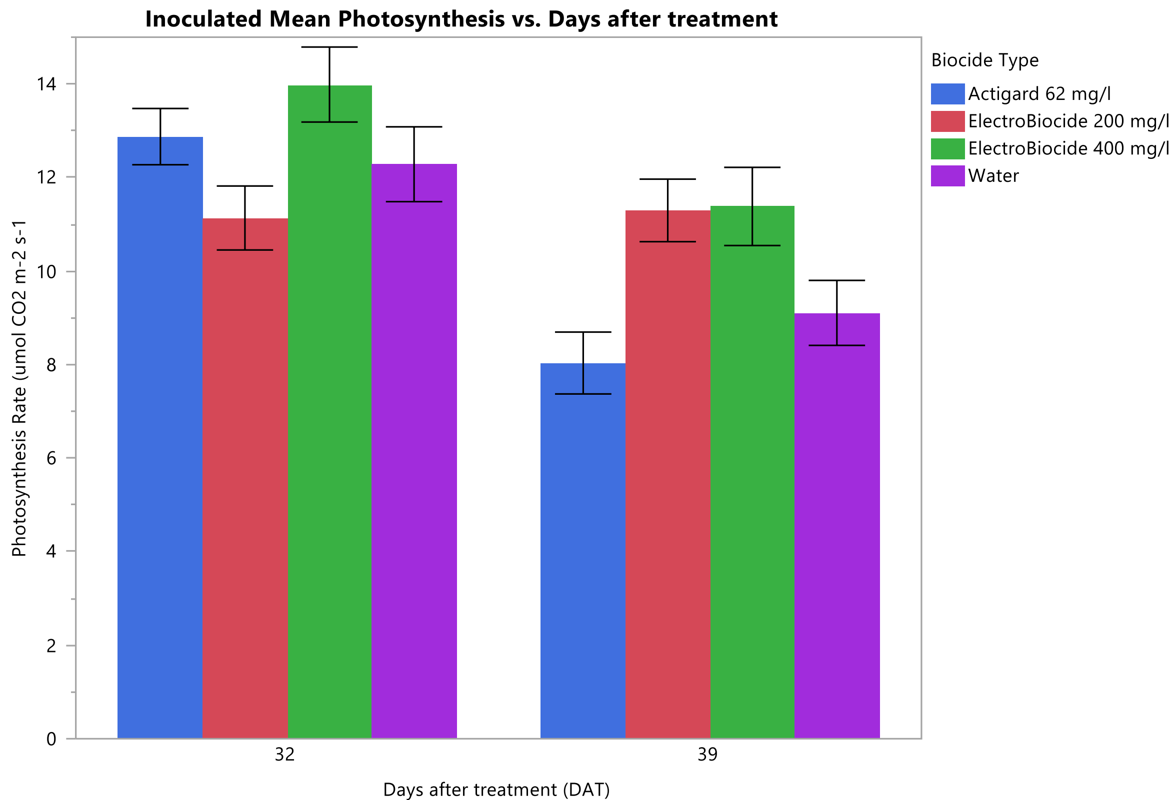

Figure 9: Inoculated Mean Photosynthesis versus Days after Treatment

Mean photosynthesis assimilation (µmol CO_2 m^{-1} $s^{-1)}$ for inoculated plants on the y axis versus days after treatment along the x axis. Actigard is represented by blue, E-B 200 mg ClO_2 l^{-1} represented by the red, E-B 400 mg ClO_2 l^{-1} represented by the green and water represented by purple. Standard error is represented by black error bars (n=12).

Table 9. Inoculated Plant Photosynthesis

Student T Test with an alpha of .05. The T test was done from the interaction days after treatment (DAT) and biocide type. The level is ordered by DAT and then followed by Biocide treatment (n=12). On the right side of the table is the least squares means for photosynthesis rates (µmol CO_2 m^{-1} s^{-1}) for inoculated plants. All levels that are not attached by the same letter are significantly different.

Level					Least Squares Mean
32,Actigard 62 mg/l	A	B			12.873921
32,Electro-Biocide 200 mg/l		B	C		11.013357
32,Electro-Biocide 400 mg/l	A				13.987241
32,Water	A	B			12.283378
39,Actigard 62 mg/l				D	8.033407
39,Electro-Biocide 200 mg/l		B			11.296144
39,Electro-Biocide 400 mg/l		B			11.381082
39,Water			C	D	9.104889

At 39 DAT Actigard and water treatments were not different than each other (Table 9). E-B 400 mg ClO_2 l^{-1} and E-B 200 mg ClO_2 l^{-1} plants had the greatest photosynthesis rate of all treatments. E-B 400 mg ClO_2 l^{-1} and E-B 200 mg ClO_2 l^{-1} were not different from each other, and resulted in a 24-25% increase when compared to the water control plants. E-B 400 mg ClO_2 l^{-1} and E-B 200 mg ClO_2 l^{-1} having greater photosynthesis rates than inoculated water treatment may indicate that the plants were dealing better with CFF infection due to priming, which resulted in being able to photosynthesize more efficiently. Overall all inoculated biocide treatment's photosynthesis showed a decrease from 32 to 39 DAT. The exception was E-B 200 mg ClO_2 l^{-1} photosynthesis rate's stayed the same from 32 DAT to 39 DAT.

3.5 Instantaneous Water Use Efficiency

There were interactions with DAT and CFF status, biocide and CFF status, and DAT and biocide when inoculated and non-inoculated plants were analyzed together for water use efficiency (WUE). At 32 and 39 DAT the inoculated and non-inoculated plants were different

from each other (Figure 10 and Table 10). Non-inoculated (all biocides combined) plants had a difference from 32 DAT measurement to 39 DAT. The inoculated plant's WUE measurements were not different from 32 to 39 DAT (Table 10). For each biocide there were differences between the inoculated and non-inoculated plants (Table 11). When plants were inoculated they had a higher WUE than their non-inoculated counter parts (Table 11). There were differences among biocides for each measurement date (Table 12). The details of each biocide at different measurement dates will be discussed more thoroughly in the next two sections where inoculated and non-inoculated plants are examined separately.

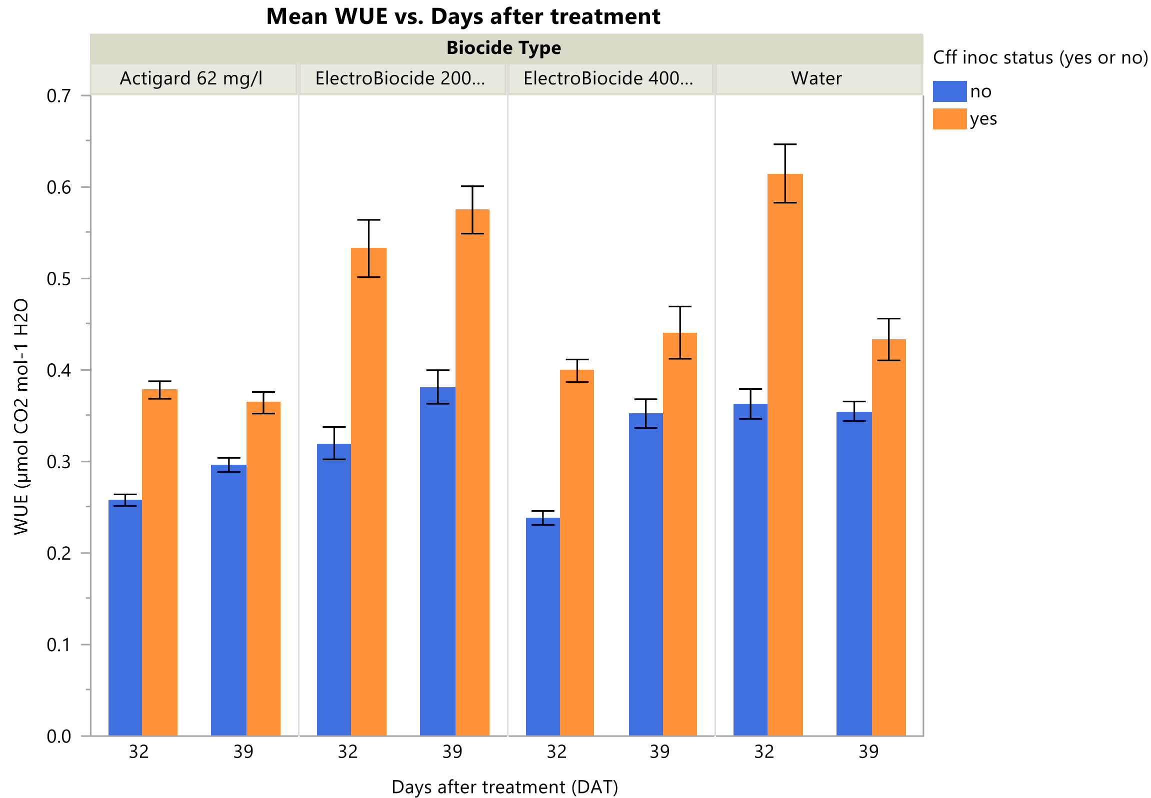

Figure 10: Mean Water Use Efficiency versus Days after Treatment

Mean instantaneous water use efficiency (WUE) (µmol CO_2 mol^{-1} H_2O) on the y axis versus days after treatment along the x axis. The graph is separated by biocide type: Actigard, E-B 200 mg ClO_2 l^{-1}, E-B 400 mg ClO_2 $l^{-1}$and water. Inoculated plants with CFF are in orange and non-inoculated plants are in blue. Standard error is represented by black error bars (n=12).

Table 10: Water Use Efficiency: Days after Treatment and CFF Status Interaction

Student T Test with an alpha of .05. The T test was done from the interaction of days after treatment (DAT) and CFF status. The level is ordered by DAT and then followed by inoculation status (yes or no) (n=12). On the right side of the table is the least squares means for water use efficiency (µmol CO_2 mol^{-1} H_2O). All levels that are not attached by the same letter are significantly different.

Level				Least Squares Mean
32,no			C	0.29480779
32,yes	A			0.48126476
39,no		B		0.34631380
39,yes	A			0.45336392

Table 11: Water Use Efficiency: Biocide Type and CFF Status Interaction

Student T Test with an alpha of .05. The T test was done from biocide type and CFF inoculation status (yes or no). The level is ordered by biocide treatment and then followed by inoculation status (n=12). On the right of table is the least squares means for water use efficiency (µmol CO_2 mol^{-1} H_2O). All levels that are not attached by the same letter are significantly different.

Level		Least Squares Mean
Actigard 62 mg/l, no	D	0.27707934
Actigard 62 mg/l, yes	C	0.37121578
Electro-Biocide 200 mg/l, no	C	0.35081161
Electro-Biocide 200 mg/l, yes	A	0.55391700
Electro-Biocide 400 mg/l, no	D	0.29538715
Electro-Biocide 400 mg/l, yes	B	0.42005516
Water, no	C	0.35896507
Water, yes	A	0.52406942

Table 12: Water Use Efficiency: Days after Treatment and Biocide Interaction

Student T Test with an alpha of .05. The T test was done from the interaction of days after treatment (DAT) and biocide type. The level is ordered by DAT and then followed by Biocide treatment (n=12). On the right of table is the least squares means of water use efficiency (µmol CO_2 mol^{-1} H_2O). All levels that are not attached by the same letter are significantly different.

Level		Least Squares Mean
32,Actigard 62 mg/l	C	0.31799496
32,Electro-Biocide 200 mg/l	B	0.42648595
32,Electro-Biocide 400 mg/l	C	0.31879084
32,Water	A	0.48887335
39,Actigard 62 mg/l	C	0.33030016
39,Electro-Biocide 200 mg/l	A	0.47824267
39,Electro-Biocide 400 mg/l	B	0.39665147
39,Water	B	0.39416115

3.5.1 Non-inoculated Plants Water Use Efficiency

At 32 DAT non-inoculated plants showed that the water treatment had the highest WUE (µmol CO_2 mol^{-1} H_20) and E-B 200 mg ClO_2 l^{-1} had the second highest (Figure 11 and Table 13). E-B 200 mg ClO_2 l^{-1} mean WUE was an 11% reduction when compared to control water. E-B 400 mg ClO_2 l^{-1} and Actigard didn't have differences in WUE from each other.

At 39 DAT non-inoculated E-B 200 mg ClO_2 l^{-1}, E-B 400 mg ClO_2 l^{-1}, and water plants were not different from each other (Table 13). Actigard WUE was lower than all other treatments at 39 DAT. Actigard, E-B 200 mg ClO_2 l^{-1} and E-B 400 mg ClO_2 l^{-1} all increased in WUE from 32 DAT to 39 DAT (Table 13). Water did not change in WUE from 32 DAT to 39 DAT.

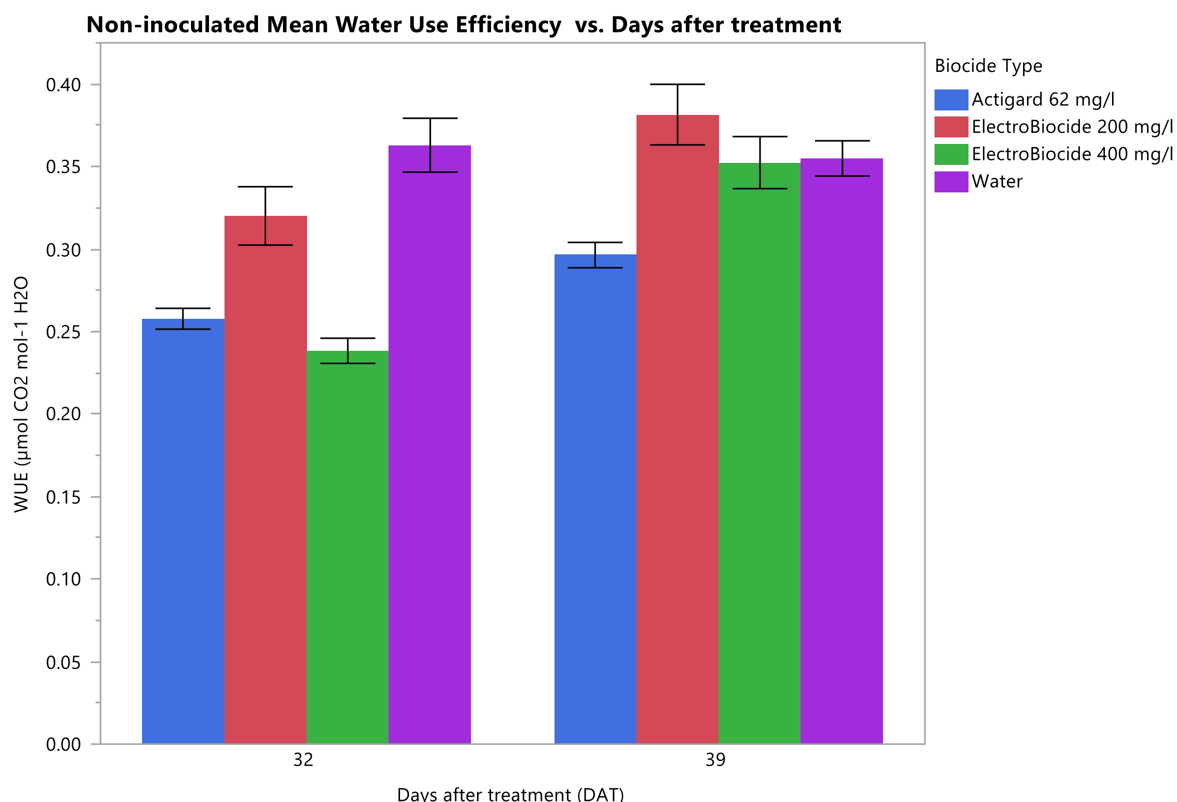

Figure 11: Non-inoculated Mean Water Use Efficiency versus Days after Treatment

Mean instantaneous water use efficiency (µmol CO_2 mol^{-1} H_2O) for non-inoculated plants on the y axis versus days after treatment along the x axis. Actigard is represented by blue, E-B 200 mg ClO_2 l^{-1} represented by the red, E-B 400 mg ClO_2 l^{-1} represented by the green and water represented by purple. Standard error is represented by black error bars (n=12).

Table 13: Non-Inoculated Water Use Efficiency

Student T Test with an alpha of .05. The T test was done from the interaction of days after treatment (DAT) and biocide type with only inoculated plants. The level is ordered by DAT and then followed by Biocide treatment (n=12). On the right side of the table is the least squares means for non-inoculated water use efficiency (μmol CO_2 mol^{-1} H_2O). All levels that are not attached by the same letter are significantly different.

Level					Least Squares Mean
32,Actigard 62 mg/l				D	0.25778835
32,Electro-Biocide 200 mg/l		B	C		0.32011496
32,Electro-Biocide 400 mg/l				D	0.23836251
32,Water	A				0.36296533
39,Actigard 62 mg/l			C		0.29637034
39,Electro-Biocide 200 mg/l	A				0.38150826
39,Electro-Biocide 400 mg/l	A	B			0.35241179
39,Water	A	B			0.35496481

3.5.2 Inoculated Plants Water Use Efficiency

At 32 DAT inoculated E-B 200 mg ClO_2 l^{-1} WUE was lower than water but was higher than Actigard and E-B 400 mg ClO_2 l^{-1}(Figure 12 and Table 14) E-B 200 mg ClO_2 l^{-1} had a 13% decrease when compared to inoculated water treatment's WUE. Actigard and E-B 400 mg ClO_2 l^{-1} were not different from each other at 32 DAT. Actigard had a 38% decrease and E-B 400 mg ClO_2 l^{-1} had a 34% decrease in WUE when compared to inoculated water treatment.

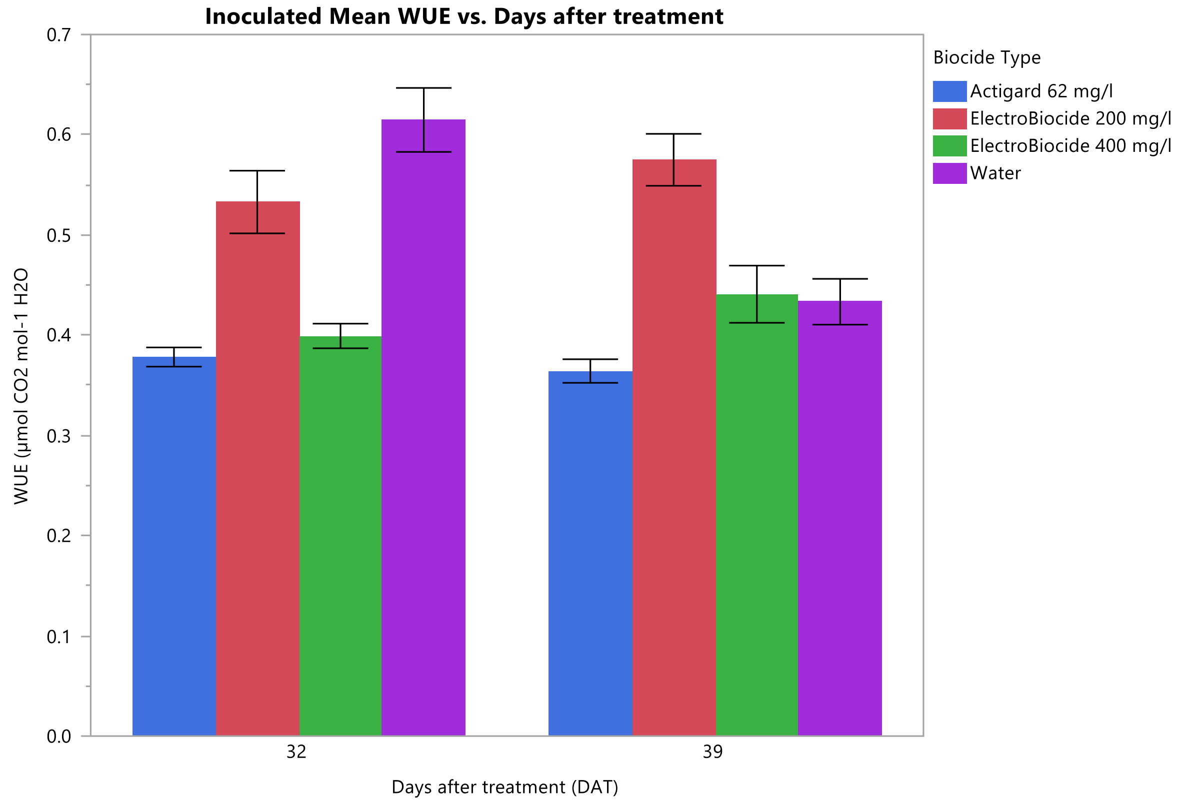

Figure 12: Inoculated Mean Water Use Efficiency versus Days after Treatment

Mean instantaneous water use efficiency (µmol CO_2 mol^{-1} H_2O) for inoculated plants on the y axis versus days after treatment along the x axis. Actigard is represented by blue, E-B 200 mg ClO_2 l^{-1} represented by the red, E-B 400 mg ClO_2 l^{-1} represented by the green and water represented by purple. Standard error is represented by black error bars (n=12).

Table 14: Inoculated Water Use Efficiency

Student T Test with an alpha of .05. The T test was done from the interaction of days after treatment (DAT) and biocide type for inoculated plants. The level is ordered by DAT and then followed by Biocide treatment (n=12). On the right side of the table is the least squares means for inoculated water use efficiency (µmol CO_2 mol^{-1} H_2O). All levels that are not attached by the same letter are significantly different.

Level					Least Squares Mean
32,Actigard 62 mg/l			C	D	0.37820158
32,Electro-Biocide 200 mg/l		B			0.54094416
32,Electro-Biocide 400 mg/l			C	D	0.39921917
32,Water	A				0.61478137
39,Actigard 62 mg/l				D	0.36422997
39,Electro-Biocide 200 mg/l	A	B			0.57497707
39,Electro-Biocide 400 mg/l			C		0.44089115
39,Water			C		0.43335748

At 39 DAT E-B 200 mg ClO_2 l^{-1} had the highest WUE (Table 14). The second highest WUE was water and E-B 400 mg ClO_2 l^{-1} which were equivalent. E-B 200 mg ClO_2 l^{-1} had a 33% increase in WUE when compared to inoculated water. The lowest WUE for inoculated plants was observed for plants treated with Actigard. Inoculated Actigard plant's WUE had a 16% decrease when compared to inoculated water control. Inoculated Actigard, E-B 200 mg ClO_2 l^{-1}, and E-B 400 mg ClO_2 l^{-1} WUE remained constant from 32 DAT to 39 DAT. Inoculated water had a decrease WUE from the first measurement at 32 DAT to 39 DAT. The increase WUE in inoculated plants is a result of a decrease in transpiration rates due to needing to conserve water (Jones, 2013). Instantaneous WUE is calculated by the ratio of photosynthesis assimilation to transpiration, which would explain why CFF inoculated plants WUE would be higher. CFF is a vascular wilt that causes a drought like stress by clogging the vascular system (Agarkova et al., 2012; Yadeta and Thomma, 2013). The plants try to conserve water due to the deficit and close their stomata. The lower stomatal conductance then conserves the needed water by transpiring less, but also reduces the photosynthesis rate. These results agree with Lorenzini et al. (1997)

which shows that the tomato vascular wilt resulted in a decrease in photosynthesis and an increase in WUE.

3.6 Stomatal Conductance

When plants were analyzed together there were interactions there were interactions with biocide and CFF status and with DAT and Biocide (Appendix II). The non-inoculated plants had a greater stomatal conductance rates than non-inoculated plants with all biocide treatments (Figure 13 and Table 15). The non-inoculated plants showed that E-B 400 mg ClO_2 l^{-1} and E-B 200 mg ClO_2 l^{-1} treatments had greater stomatal conductance than Actigard and water control treatments (when DAT combined). Inoculated plants showed that E-B 400 mg ClO_2 l^{-1} had the greatest stomatal conductance when compared to other inoculated treatments (with DAT combined).

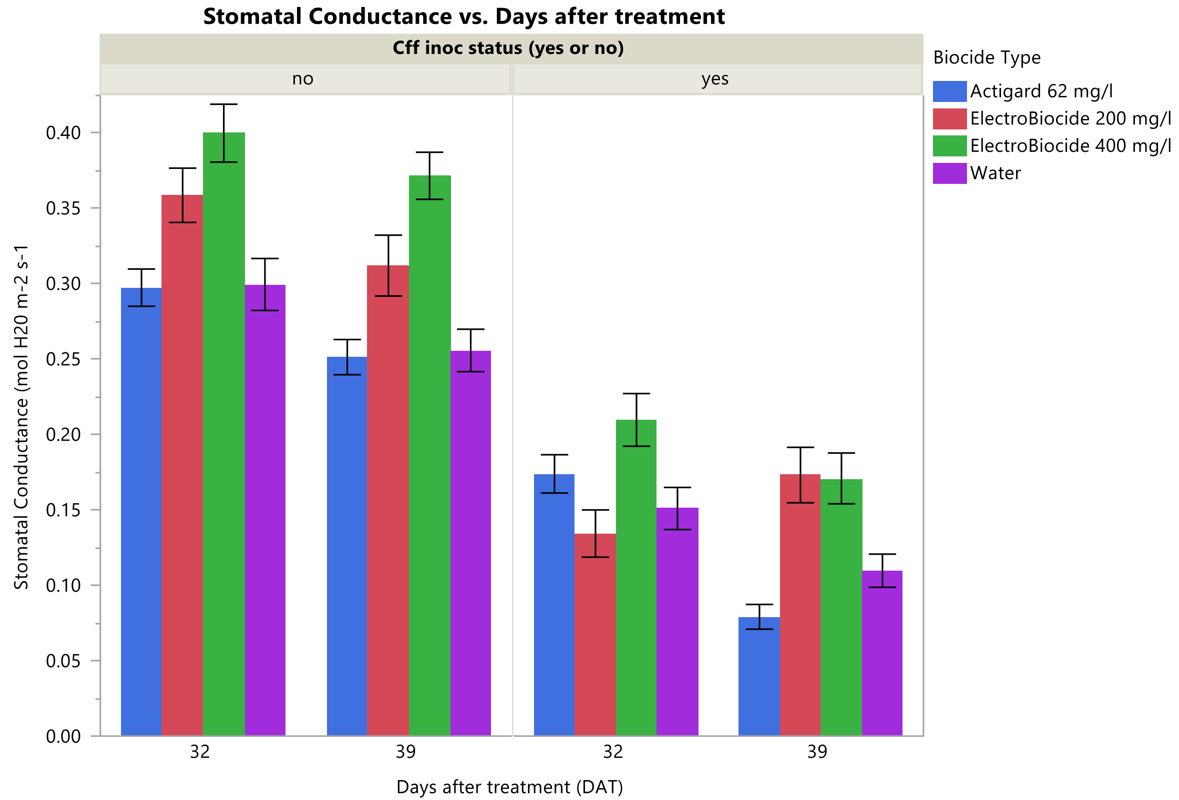

Figure 13: Mean Stomatal Conductance versus Days after Treatment

Mean stomatal conductance (mol H_2O m^{-2} s^{-1}) on the y axis versus days after treatment along the x axis. The graph is separated by CFF status with non-inoculated on the left and inoculated plants on the right. Actigard is represented by blue, E-B 200 mg ClO_2 l^{-1} represented by the red, E-B 400 mg ClO_2 l^{-1} represented by the green and water represented by purple. Standard error is represented by black error bars (n=12).

Table 15: Stomatal Conductance: Biocide and CFF Status Interaction

Student T Test with an alpha of .05. The T test was done from the interaction of CFF status and biocide type. The level is ordered by biocide treatment and then followed by inoculation status (yes or no) (n=12). On the right side of the table is the least squares means of stomatal conductance (mol H_2O m^{-2} s^{-1}). All levels that are not attached by the same letter are significantly different.

Level						Least Squares Mean
Actigard 62 mg/l, no			C			0.27447964
Actigard 62 mg/l, yes					E	0.12655575
Electro-Biocide 200 mg/l, no		B				0.33880103
Electro-Biocide 200 mg/l, yes					E	0.14937921
Electro-Biocide 400 mg/l, no	A					0.38588196
Electro-Biocide 400 mg/l, yes				D		0.19036732
Water, no			C			0.27394894
Water, yes					E	0.13036276

Actigard, E-B 400 mg ClO_2 l^{-1}, and water showed that their 32 DAT measurements for stomatal conductance were different from their 39 DAT measurements when inoculated and non-inoculated plants are combined (Table 16). E-B 200 mg ClO_2 l^{-1} did not show a difference in measurements from 32 DAT to 39 DAT (CFF status combined). DAT and CFF status will be discuss more in the inoculated and non-inoculated analysis in the next two sections.

Table 16: Stomatal Conductance: Days after Treatment and Biocide Interaction

Student T Test with an alpha of .05. The T test was done from the interaction of days after treatment (DAT) and biocide type. The level is ordered by DAT and followed by biocide treatment (n=12). On the right side of the table is the least squares means for stomatal conductance (mol H_2O m^{-2} s^{-1}). All levels that are not attached by the same letter are significantly different.

Level					Least Squares Mean
32,Actigard 62 mg/l			C		0.23575611
32,Electro-Biocide 200 mg/l		B	C		0.24550504
32,Electro-Biocide 400 mg/l	A				0.30489991
32,Water			C		0.22150900
39,Actigard 62 mg/l				D	0.16527928
39,Electro-Biocide 200 mg/l		B	C		0.24267520
39,Electro-Biocide 400 mg/l		B			0.27134938
39,Water				D	0.18280269

3.6.1 Non-inoculated Stomatal Conductance Measurements

The non-inoculated stomatal conductance analysis resulted in the DAT and biocide treatment interaction being not significant (Appendix II). DAT and biocide treatment were significant by themselves. The DAT showed that generally the (biocides combined) stomatal conductance at 32 DAT was greater than the stomatal conductance at 39 DAT (Figure 14 and Table 17). Biocides treatments showed that E-B 400 mg ClO_2 l^{-1} had the greatest stomatal conductance (Table 18). E-B 200 ClO_2 l^{-1} had the second greatest conductance. Actigard and water treated plants had the lowest stomatal conductance and were not different than each other.

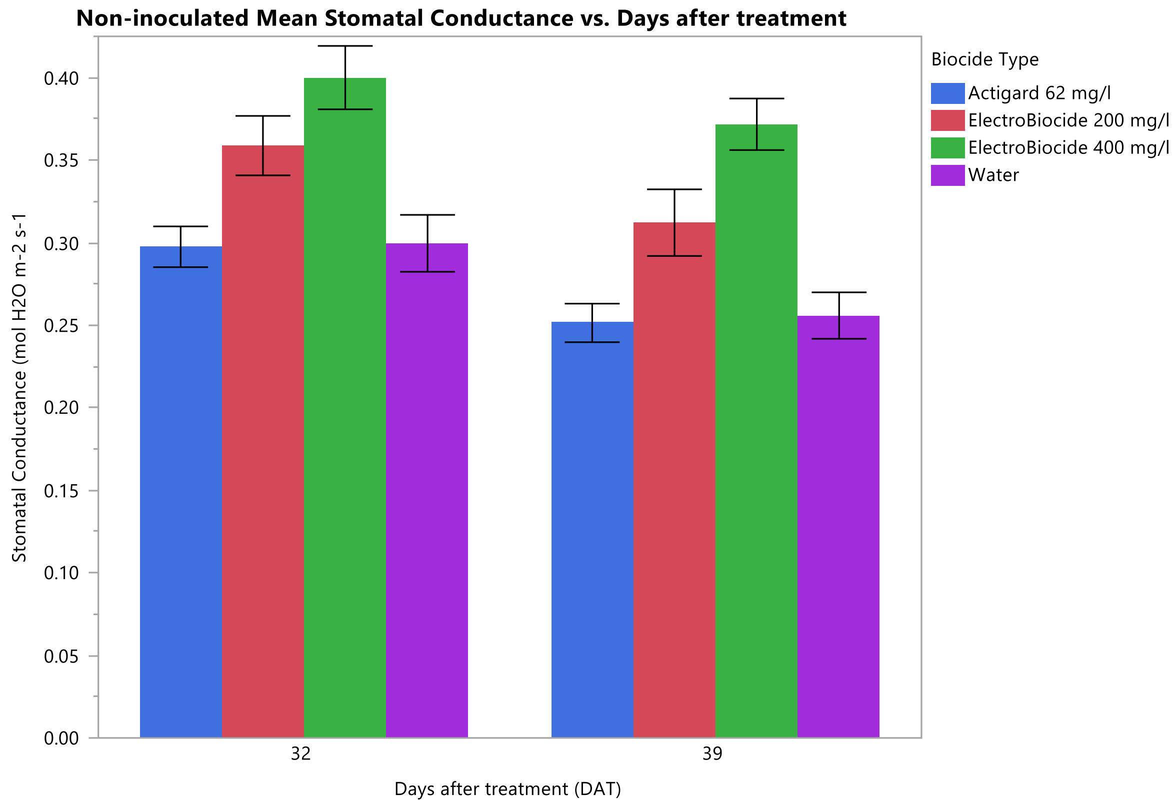

Figure 14: Non-inoculated Mean Stomatal Conductance versus Days after Treatment

Mean stomatal conductance (mol H_2O m^{-2} s^{-1}) on the y axis for non-inoculated plants with days after treatment along the x axis. Actigard is represented by blue, E-B 200 mg ClO_2 l^{-1} represented by the red, E-B 400 mg ClO_2 l^{-1} represented by the green and water represented by purple. Standard error is represented by black error bars (n=12).

Table 17: Non-inoculated Stomatal Conductance for Days after Treatment

Student T Test with an alpha of .05. The T test was done days after treatment (DAT) only (n=12). On the right side of the table is the least squares means for non-inoculated stomatal conductance (mol H_2O m^{-2} s^{-1}). All levels that are not attached by the same letter are significantly different.

Level			Least Squares Mean
32	A		0.33899984
39		B	0.29780004

Table 18: Non-inoculated Stomatal Conductance for Biocide Treatment

Student T Test with an alpha of .05. The T test was done for biocide type only (yes or no) (n=12). On the right side of the table is the least squares means of non-inoculated stomatal conductance (mol H_2O m^{-2} s^{-1}). All levels that are not attached by the same letter are significantly different.

Level				Least Squares Mean
Electro-Biocide 400 mg/l	A			0.38588196
Electro-Biocide 200 mg/l		B		0.33548712
Water			C	0.27775104
Actigard 62 mg/l			C	0.27447964

3.6.2 Inoculated Stomatal Conductance Measurements

The inoculated analysis showed that DAT and biocide did have an interaction with each other (Appendix II). At 32 DAT Actigard and E-B 400 mg ClO_2 l^{-1} were not different from each other (Figure 15 and Table 19). Both E-B 400 mg ClO_2 l^{-1} and Actigard were higher than E-B 200 mg ClO_2 l^{-1} stomatal conductance. E-B 400 mg ClO_2 l^{-1} was higher than the water control treatment plants. E-B 200 mg ClO_2 l^{-1} was equal to water control treatments. At 39 DAT the E-B 400 mg ClO_2 l^{-1} and E-B 200 mg ClO_2 l^{-1} treated plants had the highest stomatal conductance. Inoculated E-B 200 mg ClO_2 l^{-1} and E-B 400 mg ClO_2 l^{-1} had a 55% increase when compared to inoculated water control treatments. Actigard and water treatments were not different from each other. Actigard and water treatments plants showed a decrease in stomatal conductance from 32

DAT to 39 DAT. E-B 200 mg ClO_2 l^{-1} showed an increase of stomatal conductance from 32 to 39 DAT. E-B 400 mg ClO_2 l^{-1} did not change in stomatal conductance from 32 to 39 DAT.

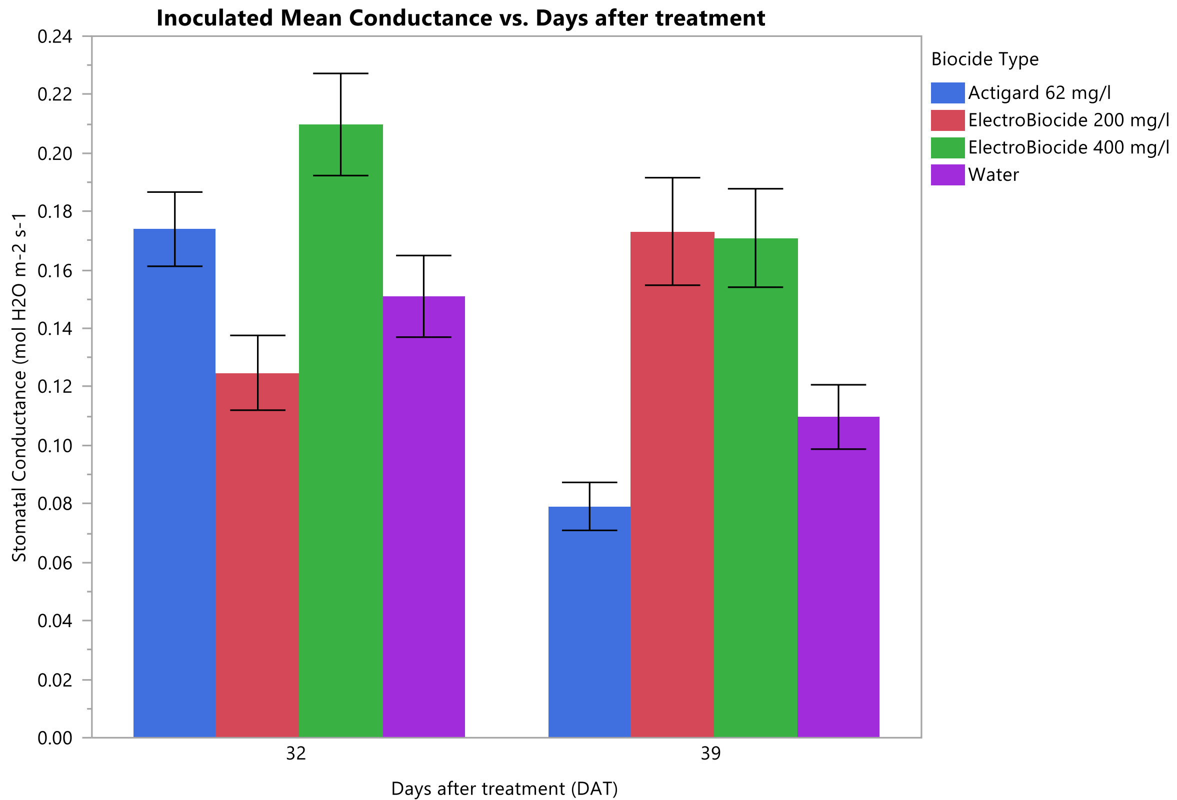

Figure 15: Inoculated Mean Stomatal Conductance versus Days after Treatment

Mean stomatal conductance (mol H_2O m^{-2} s^{-1}) on the y axis for inoculated plants with days after treatment along the x axis. Actigard is represented by blue, E-B 200 mg ClO_2 l^{-1} represented by the red, E-B 400 mg ClO_2 l^{-1} represented by the green and water represented by purple. Standard error is represented by black error bars (n=12).

Table 19: Inoculated Stomatal Conductance

Student T Test with an alpha of .05. The T test was done from the interaction of days after treatment (DAT) and biocide type. The level is ordered by DAT and then followed by biocide type (n=12). On the right side of the table is the least squares means of inoculated stomatal conductance (mol H_2O m^{-2} s^{-1}). All levels that are not attached by the same letter are significantly different.

Level						Least Squares Mean
32,Actigard 62 mg/l	A	B				0.17397599
32,Electro-Biocide 200 mg/l			C	D		0.12507648
32,Electro-Biocide 400 mg/l	A					0.20978013
32,Water		B	C			0.15099356
39,Actigard 62 mg/l					E	0.07913551
39,Electro-Biocide 200 mg/l	A	B				0.17319096
39,Electro-Biocide 400 mg/l	A	B				0.17095452
39,Water				D	E	0.10973195

3.7 Transpiration Measurements

When inoculated and non-inoculated plants were analyzed together there were interactions with DAT and CFF status, biocide type and CFF status, and DAT and biocide type (Appendix II). The inoculated and non-inoculated plants (biocides combined) were different from each other on 32 DAT and again at 39 DAT (Figure 16 and Table 20). There were also decline in transpiration rate for inoculated plants when they went from 32 to 39 DAT. The same was true for non-inoculated plants (Table 20). Each biocide's inoculated and non-inoculated transpiration rates were different from each other (Table 21). The for all biocide treatments the non-inoculated was higher in transpiration rates than their inoculated counter parts. The DAT and biocide type interaction showed that there were differences within biocides at 32 DAT and again at 39 DAT (Table 22). For each individual biocide there were differences from 32 DAT to 39 DAT. The DAT and biocide treatment will be discussed further in the next two sections for inoculated and non-inoculated analyses.

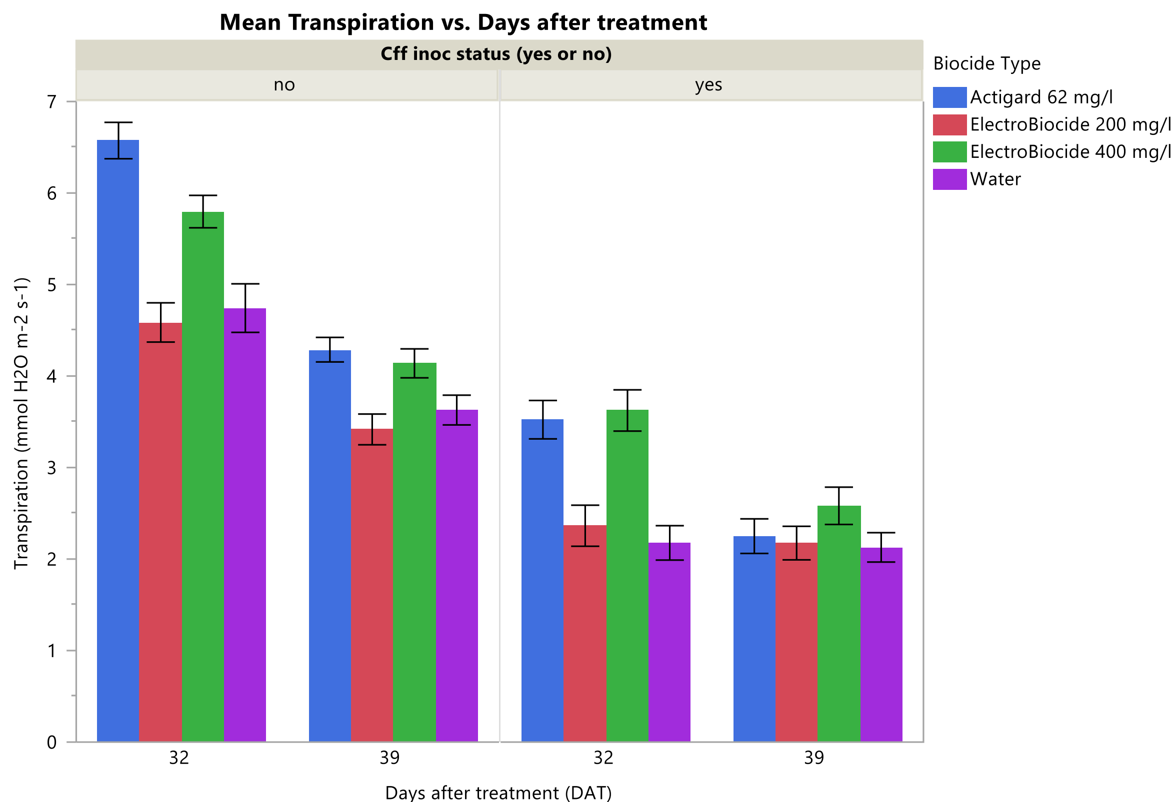

Figure 16: Mean Transpiration versus Days after Treatment

Mean transpiration rates (mmol H2O m^{-2} s^{-1}) on the y axis versus days after treatment along the x axis. The graph is separated by CFF status with non-inoculated on the left and inoculated plants on the right. Actigard is represented by blue, E-B 200 mg ClO_2 l^{-1} represented by the red, E-B 400 mg ClO_2 l^{-1} represented by the green and water represented by purple. Standard error is represented by black error bars (n=12).

Table 20: Transpiration: Days after treatment and CFF Status Interaction

Student T Test with an alpha of .05. The T test was done from the interaction of days after treatment (DAT) and CFF status (yes or no). The level is ordered by DAT and then followed by CFF status (yes or no) (n=12). On the side right of the table is the least squares means for transpiration rates (mmol H2O m^{-2} s^{-1}). All levels that are not attached by the same letter are significantly different.

Level					Least Squares Mean
32,no	A				5.4247253
32,yes			C		2.9220078
39,no		B			3.8680042
39,yes				D	2.2836996

Table 21: Transpiration: Biocide Type and CFF Status Interaction

Student T Test with an alpha of .05. The T test was done from the interaction of biocide type and CFF status. The level is ordered by biocide treatment and then followed by inoculation status (yes or no) (n=12). On the right side of the table is the least squares means for transpiration rates (mmol $H2O$ m^{-2} s^{-1}). All levels that are not attached by the same letter are significantly different.

Level						Least Squares Mean
Actigard 62 mg/l, no	A					5.4308916
Actigard 62 mg/l, yes				D		2.8867475
Electro-Biocide 200 mg/l, no			C			4.0012529
Electro-Biocide 200 mg/l, yes					E	2.2694799
Electro-Biocide 400 mg/l, no		B				4.9679009
Electro-Biocide 400 mg/l, yes				D		3.1028549
Water, no			C			4.1854136
Water, yes					E	2.1523325

Table 22: Transpiration: Days after Treatment and Biocide Interaction

Student T Test with an alpha of .05. The T test was done from the interaction of days after treatment and biocide type. The level is ordered by DAT and then followed by biocide treatment (n=12). On the right side of the table is the least squares means for transpiration rates (mmol $H2O$ m^{-2} s^{-1}). All levels that are not attached by the same letter are significantly different.

Level				Least Squares Mean
32,Actigard 62 mg/l	A			5.0482956
32,Electro-Biocide 200 mg/l		B		3.4750681
32,Electro-Biocide 400 mg/l	A			4.7104659
32,Water		B		3.4596366
39,Actigard 62 mg/l		B		3.2693435
39,Electro-Biocide 200 mg/l			C	2.7956646
39,Electro-Biocide 400 mg/l		B		3.3602899
39,Water			C	2.8781096

3.7.1 Non-Inoculated Plant's Transpiration Rates

At 32 DAT non-inoculated Actigard plants had the highest transpiration and E-B 400 mg ClO_2 l^{-1} had the second highest (Figure 17 and Table 23). When compared to the non-inoculated water mean the transpiration rates for Actigard showed a 39% increase and E-B 400 mg ClO_2 l^{-1} showed a 22% increase. Water and E-B 200 mg ClO_2 l^{-1} non-inoculated plants at 32 DAT had

lower transpirations rates when compared to Actigard and E-B 400 mg ClO_2 l^{-1}, but were not different from each other.

At 39 DAT non-inoculated Actigard and E-B 400 mg ClO_2 l^{-1} transpirations rates were not different from each other (Table 23). Actigard was higher in transpiration rates than E-B 200 mg ClO_2 l^{-1} and water control treatments. Non-inoculated water was not different from E-B 200 and E-B 400 mg ClO_2 l^{-1} plants at 39 DAT. For all non-inoculated biocide treatments there were a decrease in transpiration from 32 DAT to 39 DAT.

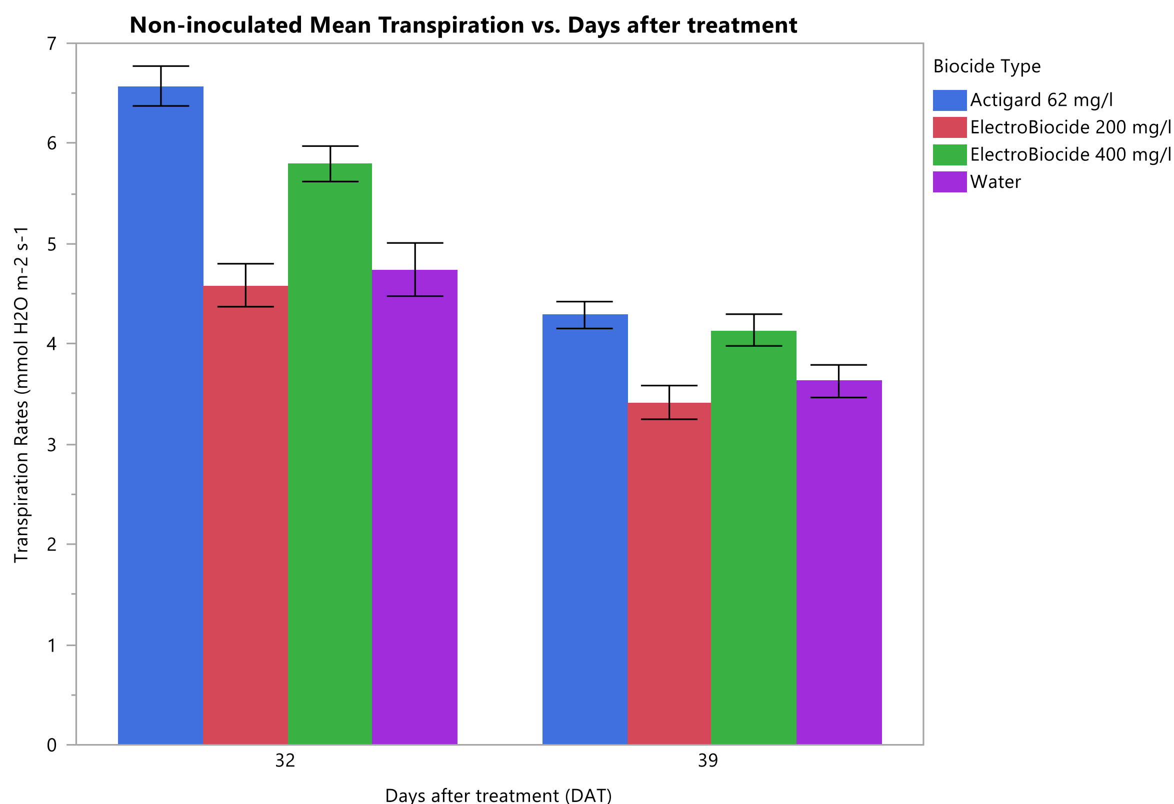

Figure 17: Non-inoculated Mean Transpiration versus Days after Treatment

Mean transpiration rates (mmol H2O m^{-2} s^{-1}) for non-inoculated plants on the y axis versus days after treatment along the x axis. Actigard is represented by blue, E-B 200 mg ClO_2 l^{-1} represented by the red, E-B 400 mg ClO_2 l^{-1} represented by the green and water represented by purple. Standard error is represented by black error bars (n=12).

Table 23: Non-inoculated Transpiration Rates

Student T Test with an alpha of .05. The T test was done from the interaction of days after treatment (DAT) and biocide type. The level is ordered by DAT and then followed by biocide treatment (n=12). On the right of table is the least squares means non-inoculated transpiration rates (mmol H2O m^{-2} s^{-1}). All levels that are not attached by the same letter are significantly different.

Level							Least Squares Mean
32,Actigard 62 mg/l	A						6.5734807
32,Electro-Biocide 200 mg/l			C	D			4.5857742
32,Electro-Biocide 400 mg/l		B					5.7971428
32,Water			C				4.7425036
39,Actigard 62 mg/l			C	D			4.2883026
39,Electro-Biocide 200 mg/l						F	3.4167315
39,Electro-Biocide 400 mg/l				D	E		4.1386590
39,Water					E	F	3.6283237

3.7.2 Inoculated Plant's Transpiration Rates

At 32 DAT Actigard and E-B 400 mg ClO_2 l^{-1} had the highest mean transpiration rates (Figure 18 and Table 24). This was a 61-66% increase when compared to inoculated water treatment. Water and E-B 200 mg ClO_2 l^{-1} inoculated plants at 32 DAT had lower transpirations rates when compared to Actigard and E-B 400 mg ClO_2 l^{-1} but were not different from each other.

At 39 DAT inoculated water, Actigard, E-B 400 mg ClO_2 l^{-1} and E-B 200 mg ClO_2 l^{-1} treatments transpiration rates were not different from each for 39 DAT. Transpiration rate for inoculated E-B 400 mg ClO_2 l^{-1} and Actigard decreased from 32 DAT to 39 DAT. Water and E-B 200 mg ClO_2 l^{-1} did not show a difference in transpiration mean from 32 DAT to 39 DAT.

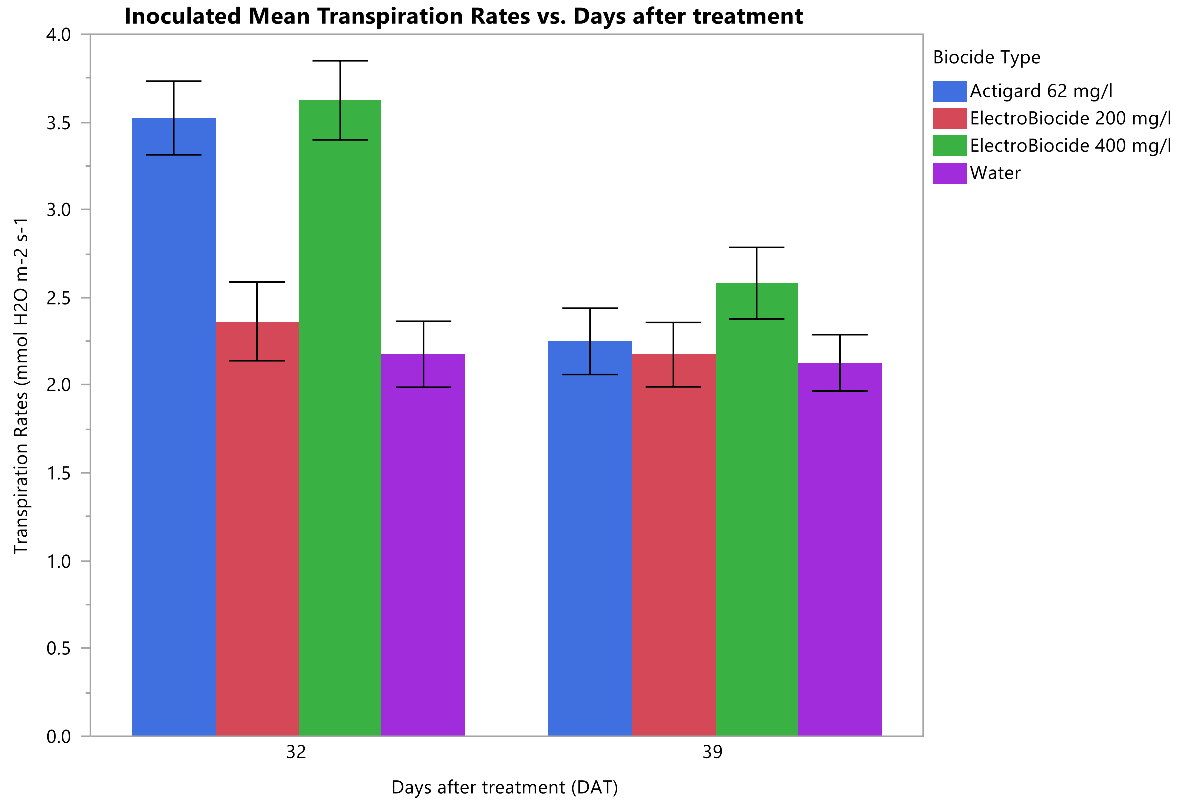

Figure 18: Inoculated Mean Transpiration versus Days after Treatment

Mean transpiration rates (mmol H2O m^{-2} s^{-1}) for inoculated plants on the y axis versus days after treatment along the x axis. Actigard is represented by blue, E-B 200 mg ClO_2 l^{-1} represented by the red, E-B 400 mg ClO_2 l^{-1} represented by the green and water represented by purple. Standard error is represented by black error bars (n=12).

Table 24: Inoculated Transpiration Rates

Student T Test with an alpha of .05. The T test was done from the interaction of days after treatment (DAT) and biocide type. The level is ordered by DAT and then followed by biocide treatment (n=12). On the right side of the table is the least squares means for inoculated transpiration rates (mmol H2O m^{-2} s^{-1}). All levels that are not attached by the same letter are significantly different.

Level			Least Squares Mean
32,Actigard 62 mg/l	A		3.5231106
32,Electro-Biocide 200 mg/l		B	2.3643620
32,Electro-Biocide 400 mg/l	A		3.6237890
32,Water		B	2.1767695
39,Actigard 62 mg/l		B	2.2503843
39,Electro-Biocide 200 mg/l		B	2.1745978
39,Electro-Biocide 400 mg/l		B	2.5819208
39,Water		B	2.1278955

3.8 Chlorophyll Fluorescence Measurements

Chlorophyll fluorescence measurements were taken 2 DAT before inoculation, 7 DAT, and 25 DAT after CFF inoculation. Data analysis revealed that soil moisture and soil temperature affected the chlorophyll fluorescence measurements (Appendix II). The fit model analysis takes out the fluctuation of the different soil temperatures and soil moisture measurements in the model. It does this by holding the soil moisture and soil temperature constant at their mean values. This adjusts any fluctuations that may have affected the other factor’s responses. There were interactions with DAT and biocide type and with DAT and CFF status. At 2 and 25 days after treatment there were no differences between inoculated and non-inoculated plants (Figure 19 and Table 25). At 7 DAT inoculated plants had higher Fv/Fm values than the non-inoculated treatments. There were differences at each measurement date when comparing all the biocide treatments (Table 26). Also, there were some differences with each biocide when comparing itself to measurements taken at 2, 7 and 25 DAT. The biocide and DAT details will be discussed further in the next two sections for the inoculated and non-inoculated analyses.

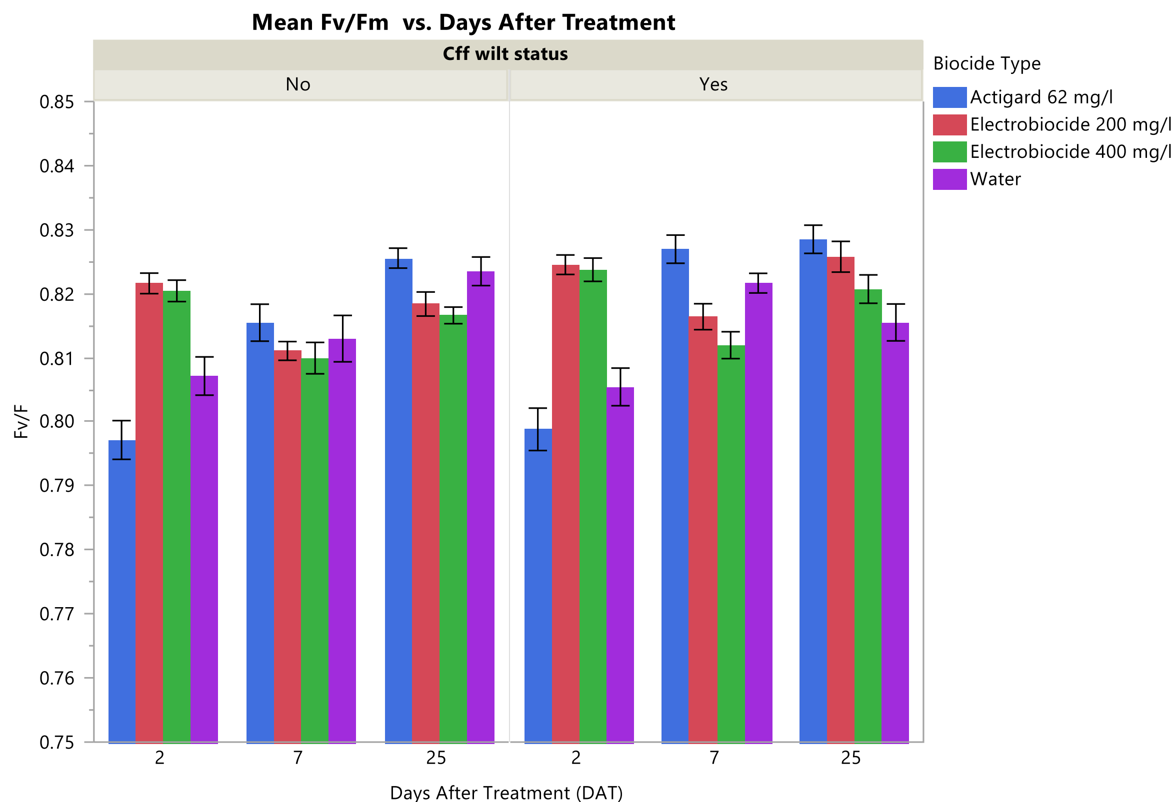

Figure 19: Mean Chlorophyll Fluorescence versus Days after Treatment

Mean Fv/Fm on the y axis versus days after treatment along the x axis. The graph is separated by CFF status with non-inoculated on the left and inoculated plants on the right. Actigard is represented by blue, E-B 200 mg ClO_2 l^{-1} represented by the red, E-B 400 mg ClO_2 l^{-1} represented by the green and water represented by purple. Standard error is represented by black error bars (n=12).

Table 25: Chlorophyll Fluorescence: Days after Treatment and CFF Status Interaction

Student T Test with an alpha of .05. The T test was done from the interaction of days after treatment (DAT) and CFF status. The level is ordered by DAT and then followed by inoculation status (yes or no) (n=12). On the right side of the table is the least squares means for Fv/Fm values. All levels that are not attached by the same letter are significantly different.

Level					Least Squares Mean
2,No				D	0.80515741
2,Yes			C	D	0.80867020
25,No	A				0.82505182
25,Yes	A				0.82461985
7,No			C		0.81073665
7,Yes		B			0.81896696

Table 26: Chlorophyll Fluorescence: Days after Treatment and Biocide Interaction

Student T Test with an alpha of .05. The T test was done from the interaction of days after treatment and biocide type. The level is ordered by days after treatment (DAT) and then followed biocide type (n=12). On the right side of the table is the least squares means for Fv/Fm values. All levels that are not attached by the same letter are significantly different.

Level							Least Squares Mean
2,Actigard 62 mg/l						F	0.79133946
2,Electrobiocide 200 mg/l	A	B					0.82556293
2,Electrobiocide 400 mg/l					E		0.80492306
2,Water					E		0.80582978
7,Actigard 62 mg/l		B	C				0.82067958
7,Electrobiocide 200 mg/l				D	E		0.81218156
7,Electrobiocide 400 mg/l					E		0.80930012
7,Water			C	D			0.81724595
25,Actigard 62 mg/l	A						0.82951305
25,Electrobiocide 200 mg/l		B	C				0.81971053
25,Electrobiocide 400 mg/l	A						0.83161688
25,Water		B	C	D			0.81850289

3.8.1 Non-inoculated Chlorophyll Fluorescence Measurements

At 2 DAT treatment E-B 200 mg ClO_2 l^{-1} had the highest Fv/Fm values (Figure 20 and Table 27). E-B 400 mg ClO_2 l^{-1} and water control treatments were not different from each other. Actigard was lower in Fv/Fm than E-B 200 mg ClO_2 l^{-1} and water control treatments. At 7 DAT Actigard, E-B 200 mg ClO_2 l^{-1}, and water control treatments were not different from each other. E-B 400 mg ClO_2 l^{-1} was only lower in Fv/Fm values than Actigard. At 25 DAT E-B 200 mg ClO_2 l^{-1}, E-B 400 mg ClO_2 l^{-1}, and water control were not different from each other. Actigard was higher than E-B 200 mg ClO_2 l^{-1}, and water control plants. Actigard and E-B 400 mg ClO_2 l^{-1} were not different from each other. The Fv/Fm values are a stress indicator of plants. The lower the values from the control the more stressed the plants are. The non-inoculated plants were showing only the effects of the spray treatments. Only Actigard was lower than the water control at 2 DAT, but recovered by 7 and 25 DAT. Both E-B treatments were always just as good in

Fv/Fm values to water control or higher. This indicates that the spray treatments for both E-B treatments did not harm the PSII and did not cause stress to the plants.

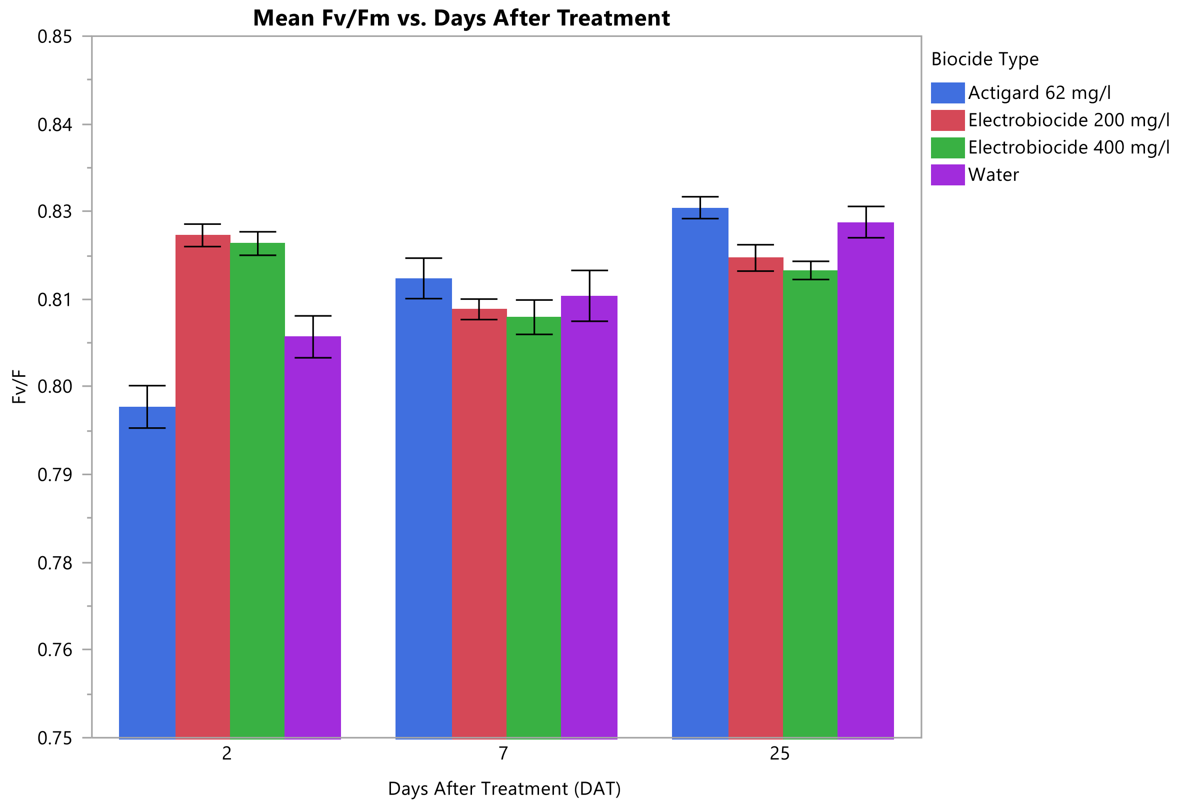

Figure 20: Mean Non-inoculated Chlorophyll Fluorescence

Mean Fv/Fm on the y axis versus days after treatment along the x axis for non-inoculated plants. Actigard is represented by blue, E-B 200 mg ClO_2 l^{-1} represented by the red, E-B 400 mg ClO_2 l^{-1} represented by the green and water represented by purple. Standard error is represented by black error bars (n=12).

Table 27: Non-inoculated Chlorophyll Fluorescence

Student T Test with an alpha of .05. The T test was done from the interaction of biocide type and days after treatment (DAT). The level is ordered by DAT and then followed by biocide treatment (n=12). On the right side of the table is the least squares means for non-inoculated Fv/Fm values. All levels that are not attached by the same letter are significantly different.

Level							Least Squares Mean
2,Actigard 62 mg/l						F	0.78881398
2,Electro-Biocide 200 mg/l	A	B					0.83188013
2,Electro-Biocide 400 mg/l			C	D	E	F	0.80308634
2,Water			C	D	E		0.80686058
7,Actigard 62 mg/l			C	D			0.81344314
7,Electro-Biocide 200 mg/l				D	E		0.80755662
7,Electro-Biocide 400 mg/l					E		0.80446612
7,Water				D	E		0.81051573
25,Actigard 62 mg/l	A						0.83096052
25,Electro-Biocide 200 mg/l		B	C	D	E		0.81164617
25,Electro-Biocide 400 mg/l	A	B					0.82708045
25,Water		B	C				0.81839483

3.8.2 Inoculated Chlorophyll Fluorescence Measurements

At 2 DAT E-B 200 mg ClO_2 l^{-1} and E-B 400 mg ClO_2 l^{-1} had the highest Fv/Fm values (Figure 21 and Table 28). This was before inoculation with CFF. This indicated there was not any damage done to PSII due to both E-B treatments at 2 DAT. Water had the second greatest Fv/Fm values and Actigard had the lowest. At 7 days after treatment Actigard was higher than both E-B treatments and was not different than water. E-B 200 mg ClO_2 l^{-1} was not different than water control treatments and E-B 400 mg ClO_2 l^{-1} was lower than water control treatment. E-B 200 mg ClO_2 l^{-1} and E-B 400 mg ClO_2 l^{-1} were not different from each other. At 25 DAT Actigard and E-B 200 mg ClO_2 l^{-1} were not different from each other and higher in Fv/Fm values than water control treatment. Actigard had greater Fv/Fm values than E-B 400 mg ClO_2 l^{-1}. E-B 200 mg ClO_2 l^{-1} was not different than E-B 400 mg ClO_2 l^{-1} at 25 DAT. E-B 400 mg ClO_2 l^{-1} and water control treatments were not different from each other.

The chlorophyll fluorescence at 7 and 25 DAT was after inoculation with CFF. E-B 400 mg ClO_2 l^{-1} was lower than water control treatment at 7 DAT indicating stress (Table 28). This was only a 1% decrease in Fv/Fm for E-B 400 mg ClO_2 l^{-1} when compared to inoculated water control treatment. The decrease in Fv/Fm was small and does not indicate a severe stress. At 25 DAT E-B 400 mg ClO_2 l^{-1} recovered and was not different than the water control. This indicates that the extra stress imposed onto E-B 400 mg ClO_2 l^{-1} treated plants at 7 DAT was temporary.

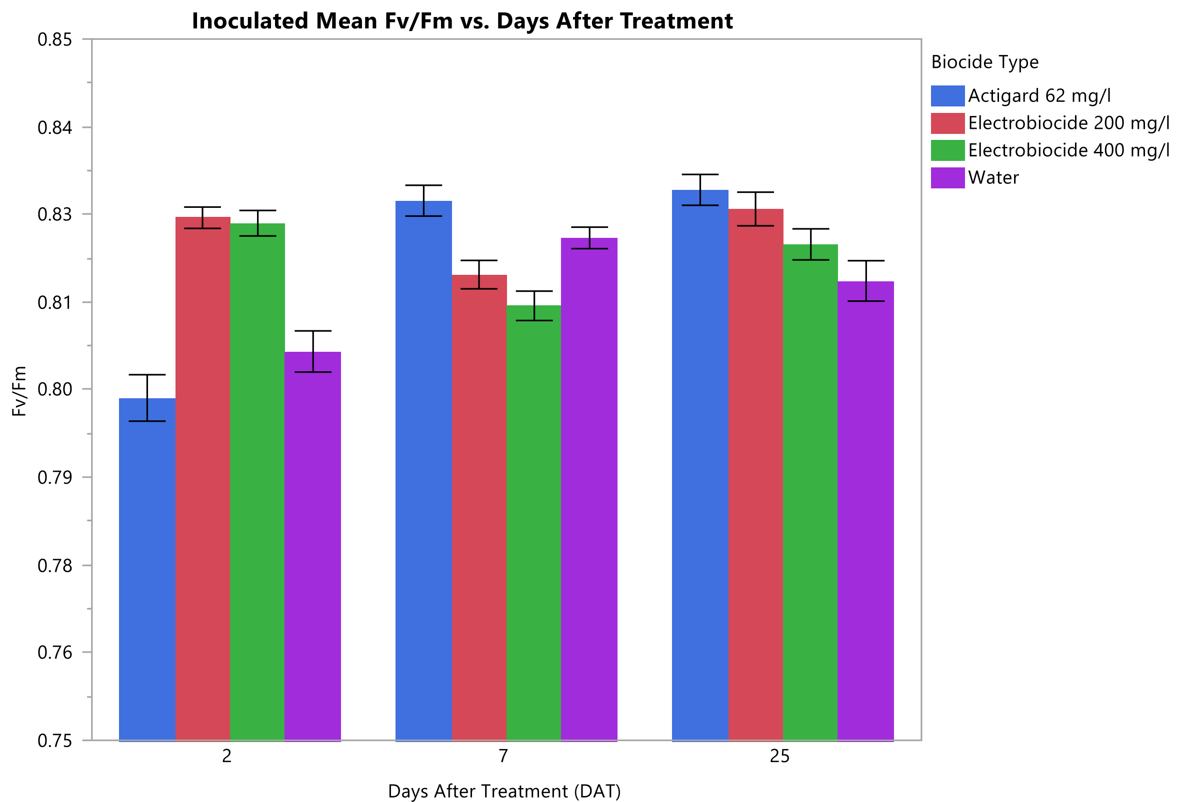

Figure 21: Mean Inoculated Chlorophyll Fluorescence

Mean Fv/Fm on the y axis versus days after treatment along the x axis for inoculated plants. Actigard is represented by blue, E-B 200 mg ClO_2 l^{-1} represented by the red, E-B 400 mg ClO_2 l^{-1} represented by the green and water represented by purple. Standard error is represented by black error bars (n=12).

Table 28: Inoculated Chlorophyll Fluorescence

Student T Test with an alpha of .05. The T test was done from the interaction of days after treatment (DAT) and biocide type. The level is ordered by DAT and then followed by biocide type (n=12). On the right side of the table is the least squares means for inoculated Fv/Fm values. All levels that are not attached by the same letter are significantly different.

Level							Least Squares Mean
2,Actigard 62 mg/l						F	0.79882508
2,Electro-Biocide 200 mg/l	A	B					0.82452403
2,Electro-Biocide 400 mg/l	A	B					0.82374042
2,Water					E		0.80544624
7,Actigard 62 mg/l	A	B					0.82695052
7,Electro-Biocide 200 mg/l			C	D			0.81640812
7,Electro-Biocide 400 mg/l				D			0.81196023
7,Water		B	C				0.82164601
25,Actigard 62 mg/l	A						0.82849401
25,Electro-Biocide 200 mg/l	A	B					0.82576723
25,Electro-Biocide 400 mg/l		B	C				0.82072044
25,Water			C	D			0.81551095

3.9 Gas Exchange and Chlorophyll Fluorescence Conclusions

Photosynthesis and chlorophyll fluorescence measurements for non-inoculated treatments were used to estimate were any priming costs with inducing SAR or oxidative stress when applying E-B 200 mg ClO_2 l^{-1} and E-B 400 mg ClO_2 l^{-1} foliar treatments. At 32 DAT there was a decline for both non-inoculated E-B 200 mg ClO_2 l^{-1} and E-B 400 mg ClO_2 l^{-1} treated plants in photosynthesis rates when compared to water control treatment. By 39 DAT the photosynthesis rates for E-B 200 mg ClO_2 l^{-1} and E-B 400 mg ClO_2 l^{-1} recovered. E-B 400 mg ClO_2 l^{-1} had a greater photosynthesis rate than control and E-B 200 mg ClO_2 l^{-1} was not different from the water control. These results reject the hypothesis that the non-inoculated E-B treatments would be just as good as the non-inoculated control in photosynthesis. The lower photosynthesis rate was only temporary and may be due to the cost of priming.

The inoculated photosynthesis rates for E-B 400 mg ClO_2 l^{-1} and E-B 200 mg ClO_2 l^{-1} was not different from inoculated water control treatment at 32 DAT. Both inoculated E-B 200 mg ClO_2 l^{-1} and 400 mg ClO_2 l^{-1} were higher than the inoculated water control at 39 DAT. These results accept the hypothesis that the inoculated plants would be just as efficient as or better than the inoculated control plants.

There was an overall decrease in photosynthesis efficiency for inoculated plants when compared to non-inoculated plants. This was due to the decrease in transpiration rates and stomatal conductance for inoculated plants. The decrease in transpiration and stomatal conductance rates are linked to conserve water due to the disease (Jones, 2013). The WUE measurements also confirm this. There was an increase WUE for inoculated plants when compared to non-inoculated. Instantaneous WUE is calculated by the ratio of photosynthesis assimilation to transpiration, which would explain why CFF inoculated plants WUE would be higher. CFF is a vascular wilt that causes a drought like stress by clogging the vascular system (Agarkova et al., 2012; Yadeta and Thomma, 2013). The plants were trying conserve water due water stress of CFF by closing their stomata. The lower transpiration and stomatal rates conserve the needed water by transpiring less, but it also reduces the photosynthesis rate. These results accept the hypothesis that the inoculated plants would be generally lower in photosynthetic measurements.

Chlorophyll fluorescence is an indicator of stress. The measurement is a ratio of variable fluorescence divided by maximum fluorescence (Fv/Fm). The lower the Fv/Fm values the more stressed the plant is due to damage to the PSII center. The chlorophyll fluorescence for both non-inoculated E-B treatments showed at 2, 7 and 25 DAT the Fv/Fm values were the same or higher

than the non-inoculated water control plants. This indicates that there was no stress due to both E-B treatments. This result accepts the hypothesis made for non-inoculated plants chlorophyll fluorescence that foliar E-B treatments would not cause stress to the plants. These results also indicate that the decline in photosynthesis was not due to photo oxidative stress due to E-B treatment.

The inoculated E-B 200 mg ClO_2 l^{-1} plants had higher Fv/Fm values than the inoculated control at 2 DAT and at 25 DAT. At 7 DAT the inoculated E-B 200 mg ClO_2 l^{-1} plants were not different than the inoculated control plants. The inoculated E-B 200 mg ClO_2 l^{-1} agrees with the hypothesis that it would have less stress than the inoculated control. Although at 7 DAT E-B 200 mg ClO_2 l^{-1} was not different from the inoculated control the two other dates E-B 200 mg ClO_2 l^{-1} showed less stress with the higher Fv/Fm values. This is especially noteworthy at 25 DAT when CFF is well established within the plant. The inoculated E-B 400 mg ClO_2 l^{-1} treatment had a higher Fv/Fm than water control at 2 DAT. At 7 DAT inoculated E-B 400 mg ClO_2 l^{-1} had a 1% decrease when compared to inoculated control, but recovered to the same Fv/Fm values of the control at 25 DAT. The hypothesis for E-B 400 mg ClO_2 l^{-1} being less stress than the inoculated control is rejected. The 7 and 25 DAT were after inoculation. E-B 400 mg ClO_2 l^{-1} resulted in being the same or lower as the inoculated control for those two dates.

Non-inoculated plants were not different in Fv/Fm values from inoculated plants at 7 DAT and 25 DAT. It is not a surprise for 2 DAT since that was before inoculation. It is surprising that at 7 DAT that the non-inoculated (all biocides combined) plants had lower Fv/Fm values than the inoculated plants. These results reject the hypothesis that all inoculated plants

would have lower Fv/Fm values (less stress) than the non-inoculated plants. Overall the Fv/Fm values indicate that there was no major decline in Fv/Fm.

3.10 Biomass

Plants were harvested 61-63 days after planting for biomass measurements. Total oven dry biomass, oven dry leaves, oven dry pod, and oven dry stems did not show differences between spray treatments (Appendix III). The only difference that was apparent for all weight measurements was that inoculated plants were much lower than non-inoculated plants (Figure 22-26). Inoculated had a 64% decrease in total above ground biomass when compared to non-inoculated plants (Figure 22 and Table 29). There was a 65% decrease in dry leaf weight in inoculated plants when compared to non-inoculated (Figure 23 and Table 30). To investigate if there was a difference in leaf water content leaves that were healthy and green were compared to oven dry green leaves. The results showed no differences to biocide types but did show that inoculated plants had a decrease in water content. Comparing non-inoculated plants to the inoculated pod oven dried weight there was a decrease of 67% decrease (Figure 24 and Table 31). When comparing inoculated dry stem weight to non-inoculated plants there was a 51% decrease (Figure 25 and Table 32).

Leaf area was measured on leaves that were green and partially green. Total leaf area did not show any biocide treatment differences but did show differences between inoculated and non-inoculated plants (Figure 26 and Table 33). When comparing to non-inoculated plants total leaf area for inoculated plants there was 86% decrease.

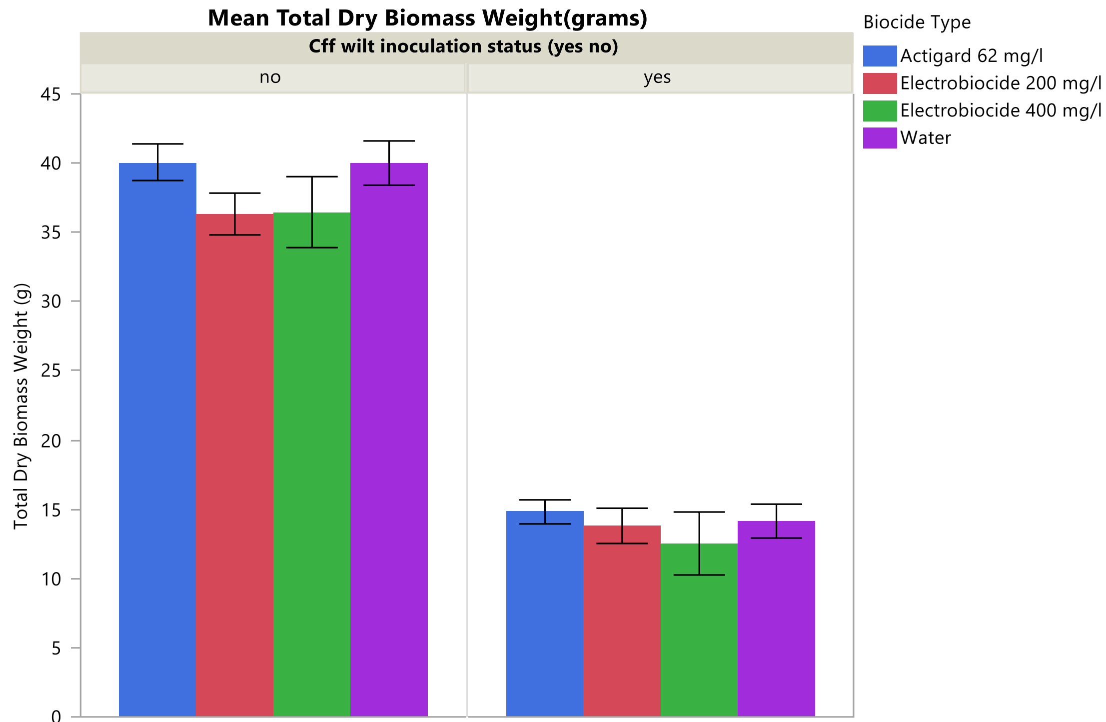

Figure 22: Mean Total Oven Dry Biomass Weight

Mean total oven biomass (g) which included leaves, stems and fruit. Total fresh biomass is on the y axis. The graph is separated by CFF status with non-inoculated on the left and inoculated plants on the right. Actigard is represented by blue, E-B 200 mg ClO_2 l^{-1} represented by the red, E-B 400 mg ClO_2 l^{-1} represented by the green and water represented by purple. Standard error is represented by black error bars (n=12).

Table 29: Total Dry Biomass Weight Table

Student T Test with an alpha of .05. The T test was for CFF status only (n=12). On the right side of the table is the least squares means for total biomass weight (g). All levels that are not attached by the same letter are significantly different.

Level			Least Squares Mean
No	A		38.215131
Yes		B	13.819765

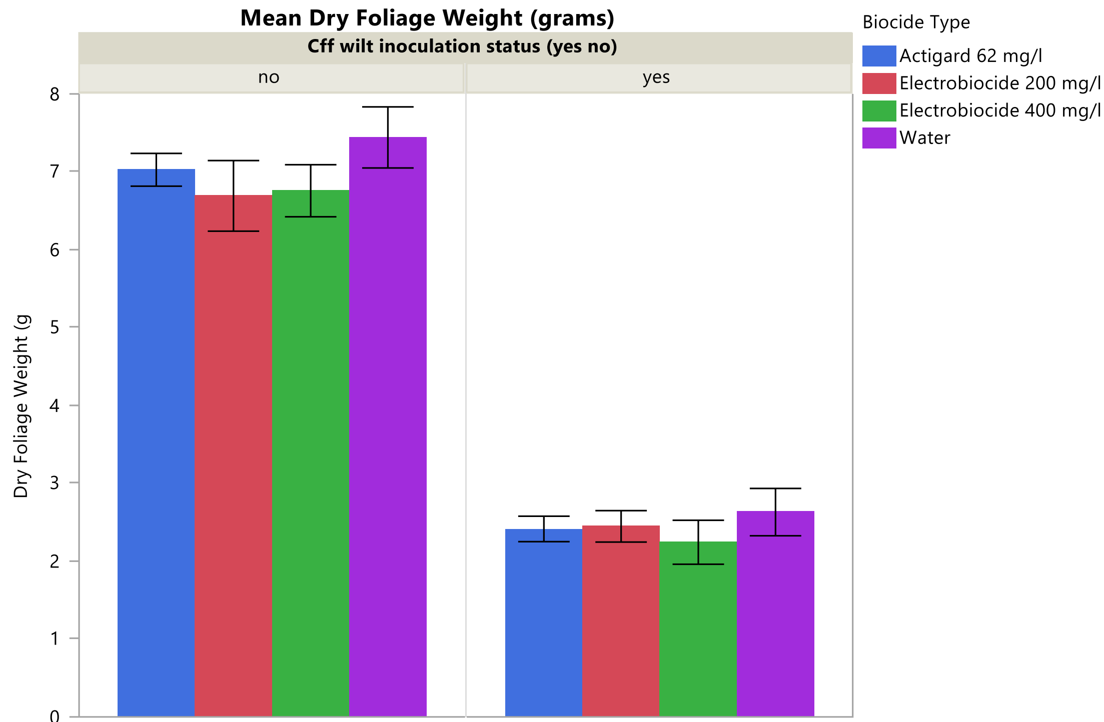

Figure 23: Mean Total Dry Foliage Weight

Mean total oven dry weight for foliage (g) on the y axis. The graph is separated by CFF status with non-inoculated on the left and inoculated plants on the right. Actigard is represented by blue, E-B 200 mg ClO_2 l^{-1} represented by the red, E-B 400 mg ClO_2 l^{-1}represented by the green and water represented by purple. Standard error is represented by black error bars (n=12).

Table 30: Dry Foliage Weight

Student T Test with an alpha of .05. The T test was for CFF status only (n=12). On the right side of the table is the least squares means for total dry foliage weight (g). All levels that are not attached by the same letter are significantly different.

Level			Least Squares Mean
No	A		6.9786122
Yes		B	2.4333902

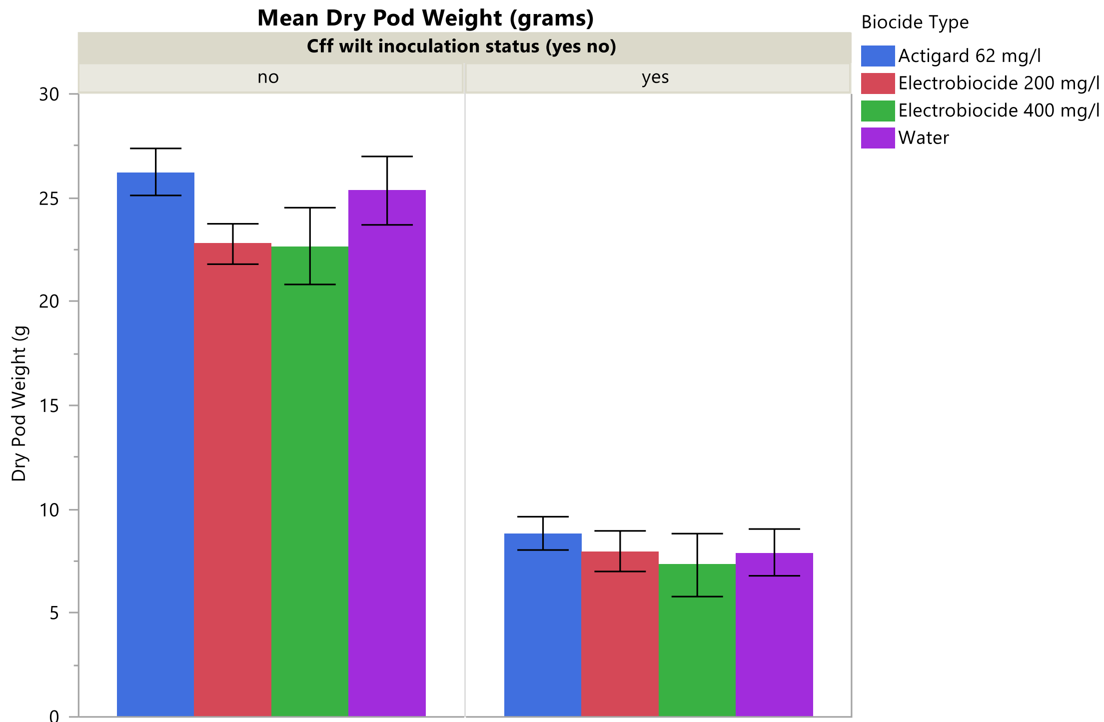

Figure 24: Mean Oven Dry Pod Weight

Mean oven dry weight of pod on the y axis. The graph is separated by CFF status with non-inoculated on the left and inoculated plants on the right. Actigard is represented by blue, E-B 200 mg ClO_2 l^{-1} represented by the red, E-B 400 mg ClO_2 l^{-1}represented by the green and water represented by purple. Standard error is represented by black error bars (n=12).

Table 31: Dry Pod Weight

Student T Test with an alpha of .05. The T test was for CFF status only (n=12). On the right side of the table is the least squares means for dry pod weight (g). All levels that are not attached by the same letter are significantly different.

Level			Least Squares Mean
no	A		24.257731
yes		B	8.012417

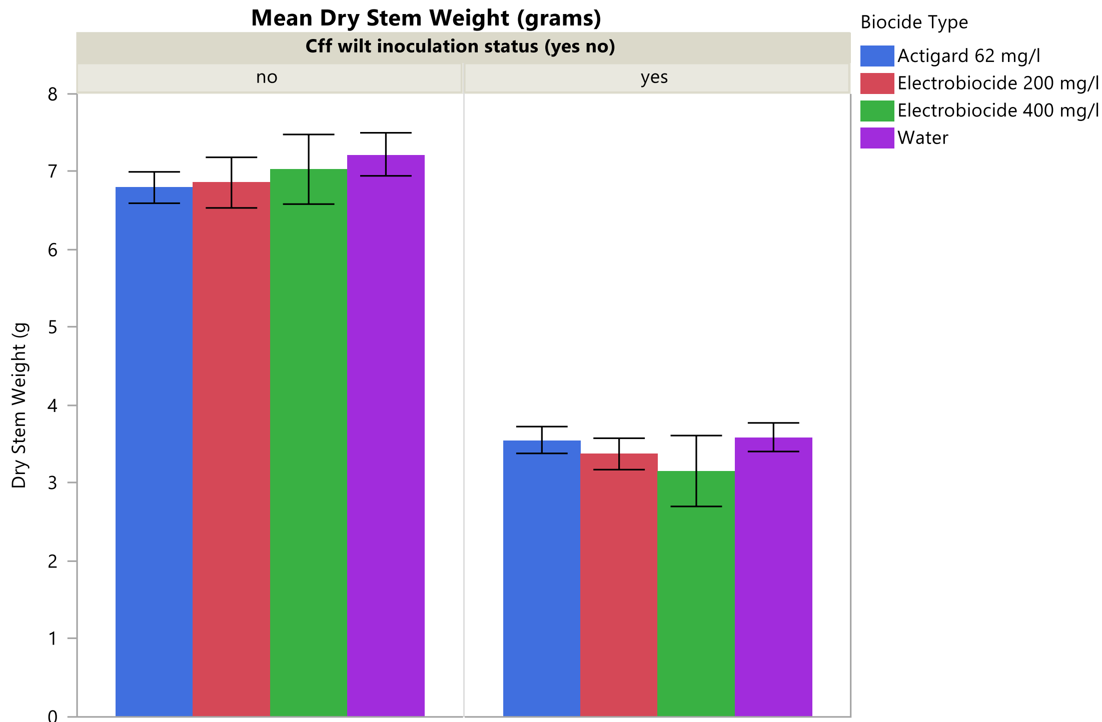

Figure 25: Mean Oven Dry Stem Weight

Mean oven dry stem weight (g) on the y axis. The graph is separated by CFF status with non-inoculated on the left and inoculated plants on the right. Actigard is represented by blue, E-B 200 mg ClO_2 l^{-1} represented by the red, E-B 400 mg ClO_2 l^{-1} represented by the green and water represented by purple. Standard error is represented by black error bars (n=12).

Table 32: Dry Stem Weight

Student T Test with an alpha of .05. The T test was for CFF status only (n=12). On the right of table is the least squares means for dry stem weight (g). All levels that are not attached by the same letter are significantly different.

Level			Least Squares Mean
no	A		6.9787885
yes		B	3.4206212

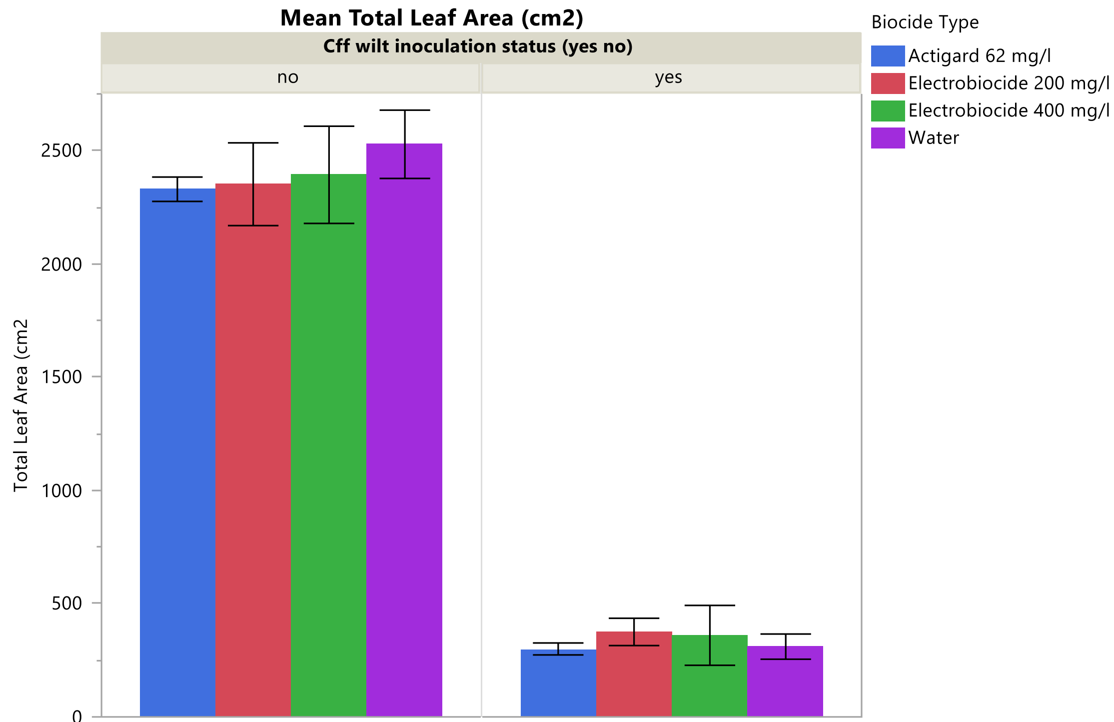

Figure 26: Mean Total Leaf Area

Mean total leaf area (cm^2) on the y axis for the whole plant using green and partially green leaves only. The graph is separated by CFF status with non-inoculated on the left and inoculated plants on the right. Actigard is represented by blue, E-B 200 mg ClO_2 l^{-1} represented by the red, E-B 400 mg ClO_2 l^{-1} represented by the green and water represented by purple. Standard error is represented by black error bars (n=12).

Table 33: Total Leaf Area

Student T Test with an alpha of .05. The T test was for CFF status only (n=12). On the right of table is the least squares means for total leaf area (cm^2). All levels that are not attached by the same letter are significantly different.

Level			Least Squares Mean
No	A		2400.2905
Yes		B	336.3424

3.11 Biomass Conclusions

The SA measurements for E-B 200 mg ClO_2 l^{-1} and E-B 400 mg ClO_2 l^{-1} resulted in rise in SA concentration which indicate SAR, but the biomass for all plants did not show a difference. Dry pod, dry leave, dry stems, dry total biomass weight and leaf area did not show any differences between biocide treatments. There was only a difference between inoculated and non-inoculated plants. These results reject the hypothesis that the E-B treated inoculated plants would have greater biomass weight than the inoculated water control plants.

Evaluating reasons why biomass may have not shown differences in different spray treatments may be due to the wilt disease having too great of disease pressure. Actigard's active ingredient ASM has been successful in numerous studies to induce SAR, but there have been a few accounts were it did not (Walters et al., 2005). This may be the case with CFF and *Phaseolus vulgaris*. The way the vascular wilts colonize the xylem and prevent water from getting to leaves may be the inhibitor for inducing a successful SAR with Actigard and E-B. When the water is stopped due to the vascular wilt clogging the xylem there is little to do to combat the wilt without the essential water. ASM has been successful at the induction of SAR and reducing disease severity in other studies. Since ASM failed to induce SAR in this study means that E-B should not be written off as ineffective in inducing SAR. Future research could explore the other pathogens and evaluate the efficiency of E-B inducing SAR. Good candidates would be plants that are foliar pathogens where plants could activate HR.

Other considerations are that the in this study plants were given all three components in disease triangle: susceptible host, right environmental conditions and the pathogen. The extreme conditions of 90 degree F and almost a 100 percent relative humidity for 48 hours was given when inoculating the plants. This ensured that the plants would successfully be inoculated with

CFF but it also put the plants at a disadvantage. A manager would implement IPM practices to avoid giving the pest ideal situations. Plants were also inoculated with needles dipped in pure cultures of CFF inoculum. In real life the concentrations of the disease would be much smaller in titer. The preliminary research done in 2012 was done with the leaf inoculation with a floral frog. That study resulted in E-B having a higher biomass weight than inoculated control. This was due to the method with leaf inoculation and with lower concentration of CFF. The plants in the preliminary research were mildly sick and were not subjected to the extreme inoculation done in this study.

Non-inoculated plants showed that there was no great priming cost by treating the plants with E-B and Actigard spray treatments. Across all plants spray treatments for non-inoculated plant dry weight and leaf area showed to be just as good as the control plants. There were no stunted plants nor great decline in pod production across all non-inoculated treatments.

3.12 PCR and CFF DNA

Polymerase chain reaction was able to confirm the prescence of CFF wilt in inoculated Actigard, E-B 200 mg ClO_2 l^{-1}, E-B 400 mg ClO_2 $l^{-1}$and water (Figure 27). The results also confirmed that non-inoculated Actigard, E-B 200 mg ClO_2 l^{-1}, E-B 400 mg ClO_2 $l^{-1}$and water were not infected with CFF wilt. Unfortunately this procedure was not able to indicate the colony forming units within each treatment. Harvesting the DNA of CFF from plant sap was not precise. Enough sap was collected onto Whatman™ FTA™ Classic Cards for the indicating paper to turn pink to white, which meant there was enough DNA to analyze. It is possible to get colony forming units through PCR, but for this study it was used it for for justifying CFF prescence or absence.

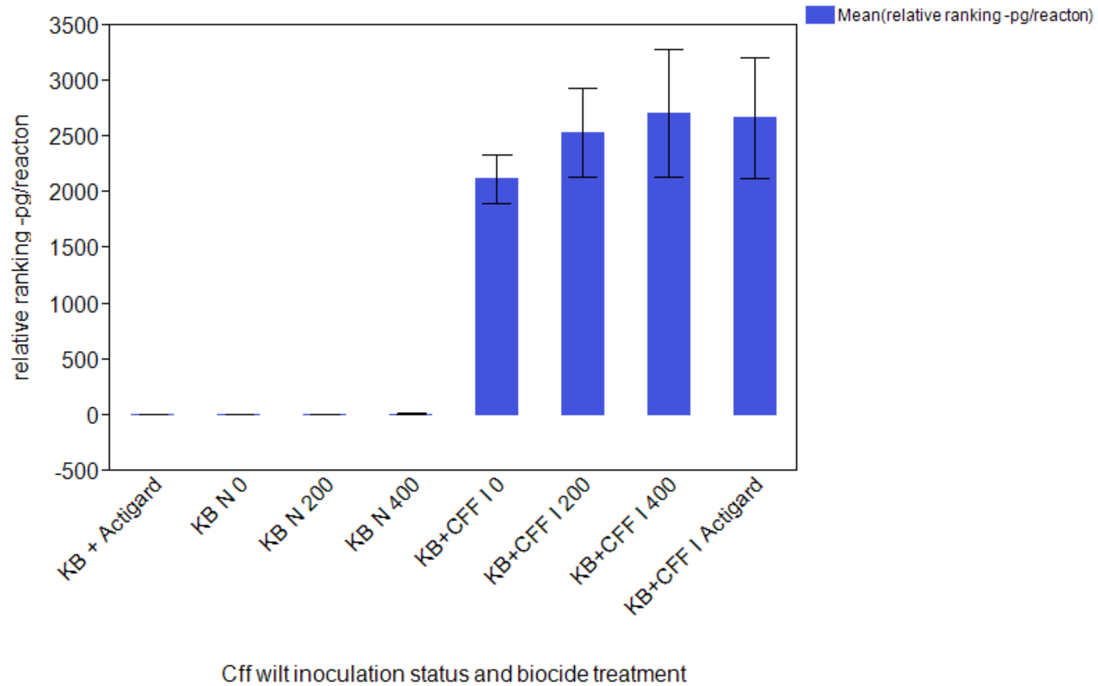

Figure 27: Mean CFF Relative Ranking versus Biocide Treatment

Results of PCR CFF wilt with relative ranking of CFF wilt status on the y axis and biocide treatments along the x axis (n=12). Biocides from left to right: Actigard non-inoculated, water non-inoculated, E-B 200 mg l^{-1} ClO_2 non-inoculated, E-B 400 mg ClO_2 l^{-1} non-inoculated, water inoculated, E-B 200 mg ClO_2 l^{-1} inoculated, E-B 400 mg ClO_2 l^{-1} inoculated, and Actigard inoculated. The KB in front of each biocide treatment stands for kidney bean.

3.13 Recommendations for Future Studies

Both E-B treatments had a rise in SA, which are crucial for SAR for reducing disease severity. SA concentrations were measured 5 DAT and had a SA response with both Free and Conjugate SA forms. This indicated that the plants were still actively producing SA 5 DAT. The second harvest (61 days after planting) at the end of the study showed that non-inoculated E-B treatments went back down to normal levels. This indicates that the E-B treatments would need multiple spray applications in order to be protected over a long period of time. Future studies can investigate when the Free and Conjugate SA concentrations go back down to normal levels and when the reapplication of E-B would be appropriate. Multiple sprays would need to be

monitored with photosynthesis and chlorophyll fluorescence to determine if there are any negative affects to the multiple sprays over a long period of time.

Both E-B treatments had a rise in SA concentrations that indicate plants were primed with a SAR response. Unfortunately none of the biomass measurements showed any differences within the inoculated biocides. Actigard (ASM) has been successful at inducing SAR and reducing disease severity, but it also did not show any differences in biomass from inoculated control. Future studies can investigate E-B (along with Actigard) with plant diseases that ASM has been successful at reducing disease severity. This would justify if E-B can be used for SAR. My recommendations for a type of disease would be a foliar disease. CFF is a vascular wilt used in this study and was too great for the plants to overcome. A foliar disease would be able to use hypersensitive response, which isolates the pathogen from spreading.

CHAPTER 4 SUMMARY

E-B foliar spray treatments show promise of being another great tool for IPM due to their dual functionality. First function prevents disease by disinfecting the surfaces of the foliage and second the chlorine dioxide interaction within the plant causes a SA response needed for SAR. For the first SA harvest E-B 200 mg ClO_2 l^{-1} and 400 mg ClO_2 l^{-1}treatments showed increase in SA concentration 5 DAT. Non-inoculated and inoculated plants with CFF did not show a difference for Free SA indicating that it was too early to have interactions. E-B 400 mg ClO_2 l^{-1} had a 15 fold increase at its highest peak and E-B 200 mg ClO_2 l^{-1} had a 5.9 fold increase at its highest peak when compared to water. Throughout collection times from 22 to 37 hours after inoculation E-B 400 mg ClO_2 l^{-1} treatment consistently had a higher concentration when compared to control. E-B 200 mg ClO_2 l^{-1} was higher than the water control treatment for all time collections. Conjugate SA for E-B 200 mg ClO_2 l^{-1} and 400 mg ClO_2 l^{-1} were also higher than water control treatments. Water had nominal Conjugate SA concentrations that showed that E-B 200 mg ClO_2 l^{-1} and E-B 400 mg ClO_2 l^{-1} were actively producing SA due to priming 5 DAT. The overall increase in SA concentration for Free SA and Conjugate SA showed that E-B was successful at priming the plant's defenses for protection against pathogens. It indicates that E-B was able to be taken in the plant to interact and elicit an SA response. Actigard did not show a difference from water for Free or Conjugate SA when compared to water. This may due to it being a functional analog to SA that replaces SA in the pathway to SAR, but does not elicit a SA response.

At the last harvest at 44 DAT (61 days after planting) there were differences in Free and Conjugate SA in inoculated versus non-inoculated. For non-inoculated plants E-B treatments

Free and Conjugate SA concentrations went down to normal levels and was not different from the water control. Proving that plants may need additional applications through the growing season in order to have protection. Inoculated plants for Free SA continued to rise for all biocide treatments. Free SA for inoculated E-B 200 mg $ClO_2\ l^{-1}$ and E-B 400 mg $ClO_2\ l^{-1}$ at the end of the study were not different from each other. Inoculated E-B 200 mg $ClO_2\ l^{-1}$ had a greater Free SA concentration than Actigard and the water control. For Conjugate SA E-B 200 mg $ClO_2\ l^{-1}$ and water had the highest concentrations. E-B 400 mg $ClO_2\ l^{-1}$ had the second highest and Actigard had the lowest. Second harvest showed that the plants were still combating the disease at with high SA concentrations.

Gas exchange measurements were taken 32 and 39 DAT. The results showed a difference between inoculated and non-inoculated plants. Stomatal conductance, photosynthesis, transpiration rates showed that inoculated plants overall had lower measurements. This is due to the CFF being a vascular wilt and clogs water in the vascular tissue. This produces a water stress like symptoms within the plant and gas exchange measurement are able to pick up on those stresses. The WUE of the inoculated plants was higher than the non-inoculated plants which was due to the need for plants to conserve water due to the wilt.

The non-inoculated plants were able to be evaluated if the priming had costs to the plant or if the E-B treatments had a negative oxidative effects with gas exchange measurements. At 32 DAT E-B 200 mg $ClO_2\ l^{-1}$ and E-B 400 mg $ClO_2\ l^{-1}$ did show a decrease in photosynthesis rates when compared to water and Actigard. E-B 200 mg $ClO_2\ l^{-1}$ and E-B 400 mg $ClO_2\ l^{-1}$ had a 14% decrease in photosynthesis when comparing to non-inoculated control. The chlorophyll fluorescence measurements confirmed that there were no damage to the PSII and that the cause

of the decrease in photosynthesis was not a result of E-B treatments. By 39 DAT E-B treatments recovered to normal photosynthesis rates when compared to water control.

Inoculated plants photosynthesis measurement showed that Actigard, E-B 200 mg ClO_2 l^{-1} and E-B 400 mg ClO_2 l^{-1} did not have a decline in photosynthesis when compared to water. They either performed equally as well or better than water control plants. At 39 DAT E-B 200 mg ClO_2 l^{-1} and E-B 400 mg ClO_2 l^{-1} had the highest photosynthesis rates when compared to Actigard and water control plants. E-B 200 mg ClO_2 l^{-1} and E-B 400 mg ClO_2 l^{-1} were not different than each other. The increase of photosynthesis rate was 24-25% when compared to water control. Actigard was not different from water at 39 DAT. These results showed that E-B treatments were able to out compete the water treatment in photosynthesis. This may be due to being primed with their treatments which could indicate that they were less stressed and better able to cope with the disease.

At the end of the study plants were harvested at 61-63 days after planting. Dry weights were taken for stems, leaves, and pods. Leaf area was taken on healthy green and paritial green leaves only. The results showed that the only major difference found was between inoculated and non-inoculated plants. Inoculated plants had a much lower weight measurements for leaves, pods, stems and leaf area. Inoculated plants had a 64% decrease in above ground total biomass when compared to non-inoculated plants. Inoculated plants did not show a difference between different spray treatments and non-inoculated plants did not show a difference between different spray treatments. Actigard has been successful at inducing SAR for lots of different diseases but did not show a decrease in disease in the case of CFF and *Phaseolus vulgaris*. There have few other studys where Actigard (ASM) was reported unsuccessful at inducing SAR. CFF is a vigorous pathogen that perliforiates within the xylem. CFF is a vascular wilt that blocks up the

vascular system and when water can't get to the leaves metabolism can't work efficiently. Since Actigard has been proven to be successful at inducing SAR in other diseases, but did not succeed with CFF proves that E-B should not be written off. There was a signficant SA accumulations that is necessary for a SAR response. Future research can utilize E-B to investigate SAR in many different pathogens. Foliar pathogens would particularly be great candidates so plants can express HR.

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APPENDIX I: SALICYLIC ACID FIT MODEL ANALYSES

Table 34: First Free SA Harvest Summary of Fit

RSquare	0.562177
RSquare Adj	0.542967
Root Mean Square Error	0.866978
Mean of Response	0.959196
Observations (or Sum Wgts)	572

Table 35: First Free SA Harvest Fixed Effect Tests

Source	**Nparm**	**DF**	**DFDen**	**F Ratio**	**Prob > F**
Leaf tissue collection time (military time-hr)	5	5	547	13.8269	<.0001*
CFF Wilt Status (Yes or No)	1	1	547	0.0777	0.7805
Biocide Type	3	3	547	182.1438	<.0001*
Leaf tissue collection time (military time-hr)*Biocide Type	15	15	547	6.2141	<.0001*

Table 36: First Conjugate SA Harvest Summary of Fit

RSquare	0.657585
RSquare Adj	0.637256
Root Mean Square Error	2.643748
Mean of Response	3.585455
Observations (or Sum Wgts)	572

Table 37: First Conjugate SA Harvest Fixed Effect Tests

Source	**Nparm**	**DF**	**DFDen**	**F Ratio**	**Prob > F**
Leaf tissue collection time (military time-hr)	5	5	539	11.4670	<.0001*
CFF Wilt Status (Yes or No)	1	1	539	0.1554	0.6936
Biocide Type	3	3	539	282.1588	<.0001*
Leaf tissue collection time (military time-hr)*CFF Wilt Status (Yes or No)	5	5	539	3.3774	0.0052*
Leaf tissue collection time (military time-hr)*Biocide Type	15	15	539	7.9731	<.0001*
CFF Wilt Status (Yes or No)*Biocide Type	3	3	539	0.2707	0.8466

Table 38: Last Harvest Free SA Summary of Fit

RSquare	0.582219
RSquare Adj	0.548986
Root Mean Square Error	2.806094
Mean of Response	3.484583
Observations (or Sum Wgts)	96

Table 39: Last Harvest Free SA Effect Tests

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
CFF Wilt Status (Yes or No)	1	1	908.35510	115.3589	<.0001*
Biocide Type	3	3	31.33838	1.3266	0.2709
CFF Wilt Status (Yes or No)*Biocide Type	3	3	25.96759	1.0993	0.3538

Table 40: Last Harvest Conjugate SA Summary of Fit

RSquare	0.770347
RSquare Adj	0.752079
Root Mean Square Error	8.896038
Mean of Response	15.29302
Observations (or Sum Wgts)	96

Table 41: Last Harvest Conjugate SA Effect Tests

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
CFF Wilt Status (Yes or No)	1	1	17776.022	224.6163	<.0001*
Biocide Type	3	3	3018.850	12.7153	<.0001*
CFF Wilt Status (Yes or No)*Biocide Type	3	3	2566.045	10.8081	<.0001*

APPENDIX II: GAS EXCHANGE FIT MODEL ANALYSES

Table 42: Photosynthesis Summary of Fit

RSquare	0.28957
RSquare Adj	0.275714
Root Mean Square Error	3.693414
Mean of Response	12.60523
Observations (or Sum Wgts)	576

Table 43: Photosynthesis Fixed Effect Tests

Source	**Nparm**	**DF**	**DFDen**	**F Ratio**	**Prob > F**
Days after treatment (DAT)	1	1	70	46.0628	<.0001*
Biocide Type	3	3	494	2.3332	0.0733
Cff inoc status (yes or no)	1	1	494	76.2104	<.0001*
Biocide Type*Cff inoc status (yes or no)	3	3	494	5.3893	0.0012*
Days after treatment (DAT)*Biocide Type	3	3	494	9.2504	<.0001*

Table 44: Non-inoculated Photosynthesis Summary of Fit

RSquare	0.061175
RSquare Adj	0.037704
Root Mean Square Error	3.164624
Mean of Response	13.94869
Observations (or Sum Wgts)	288

Table 45: Non-inoculated Photosynthesis Fixed Effect Tests

Source	**Nparm**	**DF**	**DFDen**	**F Ratio**	**Prob > F**
Days after treatment (DAT)	1	1	70	45.6743	<.0001*
Biocide Type	3	3	210	3.3281	0.0206*
Days after treatment (DAT)*Biocide Type	3	3	210	7.9979	<.0001*

Table 46: Inoculated Photosynthesis Summary of Fit

RSquare	0.156939
RSquare Adj	0.135863
Root Mean Square Error	4.329346
Mean of Response	11.26177
Observations (or Sum Wgts)	288

Table 47: Inoculated Photosynthesis Fixed Effect Test

Source	**Nparm**	**DF**	**DFDen**	**F Ratio**	**Prob > F**
Days after treatment (DAT)	1	1	70	25.9689	<.0001*
Biocide Type	3	3	210	3.8422	0.0105*
Days after treatment (DAT)*Biocide Type	3	3	210	4.1558	0.0069*

Table 48: Water Use Efficiency Summary of Fit

RSquare	0.536526
RSquare Adj	0.526648
Root Mean Square Error	0.108399
Mean of Response	0.393938
Observations (or Sum Wgts)	576

Table 49: Water Use Efficiency Fixed Effect Tests

Source	**Nparm**	**DF**	**DFDen**	**F Ratio**	**Prob > F**
Days after treatment (DAT)	1	1	70	0.8450	0.3611
Disease resistance induction agent 2	3	3	493	48.4468	<.0001*
Cff inoc status (yes or no)	1	1	493	263.9288	<.0001*
Days after treatment (DAT)*Cff inoc status (yes or no)	1	1	493	19.3182	<.0001*
Disease resistance induction agent 2*Cff inoc status (yes or no)	3	3	493	6.9124	0.0001*
Days after treatment (DAT)*Disease resistance induction agent 2	3	3	493	17.6732	<.0001*

Table 50: Non-inoculated WUE Summary of Fit

RSquare	0.277879
RSquare Adj	0.259826
Root Mean Square Error	0.080278
Mean of Response	0.320561
Observations (or Sum Wgts)	288

Table 51: Non-inoculated WUE Fixed Effect Tests

Source	**Nparm**	**DF**	**DFDen**	**F Ratio**	**Prob > F**
Days after treatment (DAT)	1	1	70	29.3471	<.0001*
Biocide Type	3	3	210	18.3014	<.0001*
Days after treatment (DAT)*Biocide Type	3	3	210	7.1851	0.0001*

Table 52: Inoculated WUE Summary of Fit

RSquare	0.432771
RSquare Adj	0.41859
Root Mean Square Error	0.131604
Mean of Response	0.467314
Observations (or Sum Wgts)	288

Table 53: Inoculated WUE Fixed Effect Test

Source	**Nparm**	**DF**	**DFDen**	**F Ratio**	**Prob > F**
Days after treatment (DAT)	1	1	70	2.0764	0.1541
Biocide Type	3	3	210	30.7483	<.0001*
Days after treatment (DAT)*Biocide Type	3	3	210	11.6076	<.0001*

Table 54: Stomatal Conductance Summary of Fit

RSquare	0.510868
RSquare Adj	0.501277
Root Mean Square Error	0.092224
Mean of Response	0.233578
Observations (or Sum Wgts)	573

Table 55: Stomatal Conductance Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Days after treatment (DAT)	1	1	70.44	24.9831	<.0001*
Biocide Type	3	3	492.2	29.1076	<.0001*
Cff inoc status (yes or no)	1	1	492.3	481.6692	<.0001*
Biocide Type*Cff inoc status (yes or no)	3	3	492.2	3.1038	0.0263*
Days after treatment (DAT)*Biocide Type	3	3	492.2	3.2250	0.0224*

Table 56: Non-Inoculated Stomatal Conductance Summary of Fit

RSquare	0.119839
RSquare Adj	0.097677
Root Mean Square Error	0.0986
Mean of Response	0.318293
Observations (or Sum Wgts)	286

Table 57: Non-inoculated Stomatal Conductance Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Days after treatment (DAT)	1	1	70.34	16.1990	0.0001*
Biocide Type	3	3	209.5	21.8672	<.0001*
Days after treatment (DAT)*Biocide Type	3	3	209.5	0.2301	0.8754

Table 58: Inoculated Stomatal Conductance Summary of Fit

RSquare	0.06969
RSquare Adj	0.046349
Root Mean Square Error	0.088158
Mean of Response	0.149159
Observations (or Sum Wgts)	287

Table 59: Inoculated Stomatal Conductance Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Days after treatment (DAT)	1	1	69.96	11.8838	0.0010*
Biocide Type	3	3	209.6	7.9127	<.0001*
Days after treatment (DAT)*Biocide Type	3	3	209.6	8.0397	<.0001*

Table 60: Transpiration Summary of Fit

RSquare	0.576613
RSquare Adj	0.567589
Root Mean Square Error	1.152764
Mean of Response	3.624609
Observations (or Sum Wgts)	576

Table 61: Transpiration Fixed Effect Tests

Source	**Nparm**	**DF**	**DFDen**	**F Ratio**	**Prob > F**
Days after treatment (DAT)	1	1	70	116.2631	<.0001*
Biocide Type	3	3	493	32.5511	<.0001*
Cff inoc status (yes or no)	1	1	493	452.5179	<.0001*
Days after treatment (DAT)*Cff inoc status (yes or no)	1	1	493	22.8506	<.0001*
Biocide Type*Cff inoc status (yes or no)	3	3	493	3.4294	0.0170*
Days after treatment (DAT)*Biocide Type	3	3	493	8.7526	<.0001*

Table 62: Non-inoculated Transpiration Summary Fit

RSquare	0.478602
RSquare Adj	0.465567
Root Mean Square Error	1.113701
Mean of Response	4.646365
Observations (or Sum Wgts)	288

Table 63: Non-inoculated Transpiration Fixed Effect Tests

Source	**Nparm**	**DF**	**DFDen**	**F Ratio**	**Prob > F**
Days after treatment (DAT)	1	1	70	124.0510	<.0001*
Biocide Type	3	3	210	26.0740	<.0001*
Days after treatment (DAT)*Biocide Type	3	3	210	4.2915	0.0058*

Table 64: Inoculated Transpiration Summary of Fit

RSquare	0.063585
RSquare Adj	0.040174
Root Mean Square Error	1.234803
Mean of Response	2.602854
Observations (or Sum Wgts)	288

Table 65: Inoculated Transpiration Fixed Effect Tests

Source	**Nparm**	**DF**	**DFDen**	**F Ratio**	**Prob > F**
Days after treatment (DAT)	1	1	70	25.8904	<.0001*
Biocide Type	3	3	210	10.1479	<.0001*
Days after treatment (DAT)*Biocide Type	3	3	210	4.3836	0.0051*

Table 66: Chlorophyll Fluorescence Summary of Fit

RSquare	0.333106
RSquare Adj	0.315535
Root Mean Square Error	0.013518
Mean of Response	0.816705
Observations (or Sum Wgts)	858

Table 67: Chlorophyll Fluorescence Fixed Effect Tests

Source	**Nparm**	**DF**	**DFDen**	**F Ratio**	**Prob > F**
Days After Treatment (DAT)	2	2	284.3	15.2480	<.0001*
Biocide Type	3	3	761.5	4.9206	0.0022*
Cff wilt status	1	1	766.7	12.4235	0.0004*
soil temperature	1	1	832.6	5.5807	0.0184*
soil moisture	1	1	829.4	0.0010	0.9746
Days After Treatment (DAT)*Biocide Type	6	6	782.8	13.9882	<.0001*
Days After Treatment (DAT)*Cff wilt status	2	2	749.3	6.5405	0.0015*
Days After Treatment (DAT)*soil moisture	2	2	830.1	8.2695	0.0003*
Biocide Type*soil temperature	3	3	817.3	4.8956	0.0022*
soil temperature*soil moisture	1	1	816.9	13.4837	0.0003*

Table 68: Non-inoculated Chlorophyll Fluorescence Summary of Fit

RSquare	0.332669
RSquare Adj	0.301439
Root Mean Square Error	0.01339
Mean of Response	0.815054
Observations (or Sum Wgts)	426

Table 69: Non-inoculated Chlorophyll Fluorescence Fixed Effect Tests

Source	**Nparm**	**DF**	**DFDen**	**F Ratio**	**Prob > F**
Days After Treatment (DAT)	2	2	268	10.0944	<.0001*
Biocide Type	3	3	345.2	3.3253	0.0199*
soil temperature	1	1	404	0.5714	0.4501
soil moisture	1	1	396.3	0.5110	0.4751
Days After Treatment (DAT)*Biocide Type	6	6	360.1	6.3808	<.0001*
Days After Treatment (DAT)*soil moisture	2	2	395.5	3.3975	0.0344*
Biocide Type*soil temperature	3	3	398.4	4.1288	0.0067*
soil temperature*soil moisture	1	1	397.3	4.7616	0.0297*

Table 70: Inoculated Chlorophyll Fluorescence Summary of Fit

RSquare	0.371585
RSquare Adj	0.355127
Root Mean Square Error	0.013461
Mean of Response	0.818333
Observations (or Sum Wgts)	432

Table 71: Inoculated Chlorophyll Fluorescence Fixed Effect Tests

Source	**Nparm**	**DF**	**DFDen**	**F Ratio**	**Prob > F**
Days After Treatment (DAT)	2	2	105	14.3376	<.0001*
Biocide Type	3	3	315	6.4704	0.0003*
Days After Treatment (DAT)*Biocide Type	6	6	315	20.9663	<.0001*

APPENDIX III: BIOMAS FIT MODEL ANALYSES

Table 72: Total above Ground Biomass Summary of Fit

RSquare	0.828809
RSquare Adj	0.815192
Root Mean Square Error	5.813053
Mean of Response	26.26677
Observations (or Sum Wgts)	96

Table 73: Total above Ground Biomass Fixed Effect Tests

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Biocide Type	3	3	152.384	1.5032	0.2193
Cff wilt inoculation status (yes no)	1	1	14258.286	421.9478	<.0001*
Biocide Type*Cff wilt inoculation status (yes no)	3	3	39.678	0.3914	0.7595

Table 74: Dry Foliage Weight Summary of Fit

RSquare	0.832239
RSquare Adj	0.818895
Root Mean Square Error	1.068944
Mean of Response	4.753021
Observations (or Sum Wgts)	96

Table 75: Dry Foliage Weight Fixed Effect Tests

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Biocide Type	3	3	4.07721	1.1894	0.3185
Cff wilt inoculation status (yes no)	1	1	494.95174	433.1642	<.0001*
Biocide Type*Cff wilt inoculation status (yes no)	3	3	1.00198	0.2923	0.8309

Table 76: Leaf Water Content Summary of Fit

RSquare	0.847377
RSquare Adj	0.835236
Root Mean Square Error	6.248613
Mean of Response	17.2344
Observations (or Sum Wgts)	96

Table 77: Leaf Water Content Fixed Effect Tests

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Biocide Type	3	3	12.499	0.1067	0.9560
Cff wilt inoculation status (yes no)	1	1	19000.115	486.6190	<.0001*
Biocide Type*Cff wilt inoculation status (yes no)	3	3	23.505	0.2007	0.8957

Table 78: Dry Pod Weight Summary of Fit

RSquare	0.779663
RSquare Adj	0.762136
Root Mean Square Error	4.543624
Mean of Response	16.2951
Observations (or Sum Wgts)	96

Table 79: Dry Pod Weight Fixed Effect Tests

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Biocide Type	3	3	98.3100	1.5873	0.1981
Cff wilt inoculation status (yes no)	1	1	6322.7917	306.2697	<.0001*
Biocide Type*Cff wilt inoculation status (yes no)	3	3	33.6372	0.5431	0.6541

Table 80: Dry Stem Weight Summary of Fit

RSquare	0.758919
RSquare Adj	0.739742
Root Mean Square Error	1.050361
Mean of Response	5.240063
Observations (or Sum Wgts)	96

Table 81: Dry Stem Weight Fixed Effect Tests

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Biocide Type	3	3	1.47267	0.4449	0.7215
Cff wilt inoculation status (yes no)	1	1	303.32302	274.9339	<.0001*
Biocide Type*Cff wilt inoculation status (yes no)	3	3	1.26265	0.3815	0.7666

Table 82: Total Leaf Area Summary of Fit

RSquare	0.851635
RSquare Adj	0.839833
Root Mean Square Error	450.5962
Mean of Response	1389.492
Observations (or Sum Wgts)	96

Table 83: Total Leaf Area Fixed Effect Tests

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Biocide Type	3	3	132860	0.2181	0.8836
Cff wilt inoculation status (yes no)	1	1	102058734	502.6610	<.0001*
Biocide Type*Cff wilt inoculation status (yes no)	3	3	200426	0.3290	0.8044