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Carbon Dioxide Supplementation to Enhance Agricultural Production via Foliar Misting

Mariana Heredia Mendez
mherediamendez@stcloudstate.edu

Carbon Dioxide Supplementation to Enhance Agricultural Production via Foliar Misting

by

Mariana Heredia Mendez

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Thesis Committee:
Matthew L. Julius, Chairperson
Jorge E. Arriagada
Debra K. Japp

Abstract

Technological advances have allowed for increased accessibility and production of many goods to our growing world population. Food production is an area of increased interest; with research focusing on both increases in productivity and enhancing sustainability. Plant growth is a function of light, temperature, and nutrient availability. Carbon is a macronutrient acquired from the atmosphere and converted to carbohydrates in the leaves for delivery to other plant tissue regions. In this research we examine the use of CO_2 infused foliar spray as a mechanism for enhancing CO_2 delivery to leaves. This methodology enhances plant group while decreasing the use of chemical fertilizers and increasing carbon sequestration efficiency. This work demonstrates physiological modifications in plant biochemistry and growth consistent with CO_2 uptake by the organisms. This evidence includes chlorophyll A enhancement and increases in biomass, maturation time, and fruit production. Research also identifies optimum design foliar mist delivery to target organism. An additional benefit of the application is also documented. The when CO2 is added to water the pH decreases. As the plant removes the CO2 from water for carbon assimilation, the pH increases. This pH instability in the microenvironment makes it difficult for certain bacterial and fungal micropathogens to establish themselves on the plant. Results of this work should be beneficial to growers in enclosed growing environments and urban situations.

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Chapter I: Introduction

All life on earth requires an energy source to maintain metabolic activities, and much of life requires oxygen to retrieve stored energy for these chemical processes. Ultimately, the sun supplies the energy for the bulk of earths' life. Light energy from the sun is converted to bioavailable chemical energy via photosynthesis. This conversion takes place as a two-step process, known as the light and light independent reactions (Bryant & Frigaard 2006). These steps can be summarized with the following equation: $6CO_2 + 12H_2O + \text{light} \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O$.

As noted in the equation, the process requires light (energy), carbon dioxide, and water. Energy is stored as the sugar glucose, short-term, which may be latter converted into carbohydrates or lipids for long-term storage. "Waste" products include oxygen and water (Raven et al. 2005). The light reactions involve the initial conversion of light energy to chemical energy. In this step, water and light energy are used to produce the energy rich molecules for use in assembling glucose. This is accomplished when light excites a chlorophyll A molecule and an electron transport chain is used for converting energy poor ADP and NADP to energy rich ATP and NADPH (Berg et al. 2002).

Glucose is assembled during the second photosynthetic stage. This phase is known as the Calvin Cycle, Dark Cycle, or Light Independent Cycle. ATP and NADPH are used to fuel a metabolic pathway that cleave carbon and oxygen from carbon dioxide molecules. The oxygen is released as waste and the protein ribulose 1, 5 bisphosphate (rubisco) carries the carbon molecule through an iterative biochemical pathway ultimately linking six carbon atoms together to form glucose (Bassham et al. 1950).

Photosynthesis is typically limited by three environmental factors; light, temperature, and carbon-dioxide (Kirschbaum 2011) (Figure 1). Understanding and adjusting for these limitations is critical for commercial horticulture, which ultimately sustains food production for humans globally (Ort et al. 2015). Temperature is, perhaps, the simplest of these factors to control and can easily be regulated in most greenhouse environments. Light has historically been more difficult to control, because of regional day length restrictions and the costs of artificial lighting. Recent advances in LED technologies have helped overcome these difficulties (Tewolde et al. 2018) allowing cost effective delivery of red (662 nm) and blue (430 nm) light to stimulate chlorophyll A in the light reactions (Balegh & Biddulph 1970).

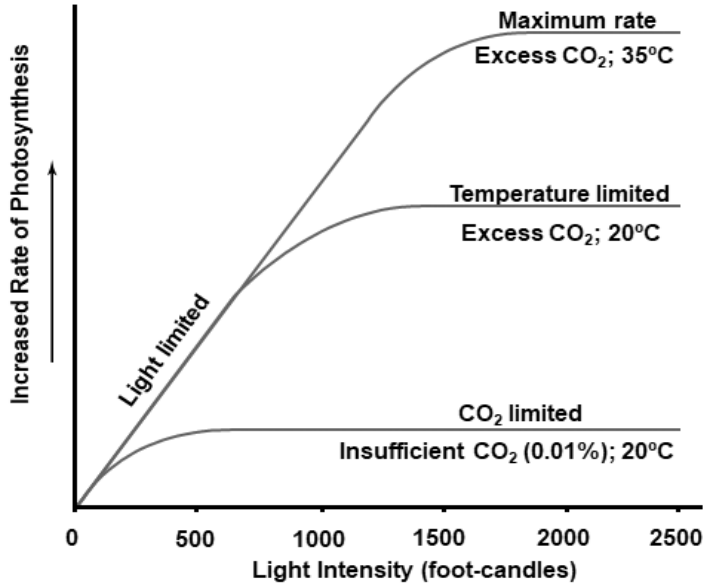

Figure 1. Photosynthetic activity at two temperature and three CO_2 levels across multiple light spectra. Modified from Farquhar et al. 1980.

Unlike temperature and light, augmenting CO_2 delivery to plant leaves is not a simple process. CO_2 does not comprise a major portion of the earth's atmosphere. Even with the considerable anthropogenic addition of CO_2 to the atmosphere via fossil fuels, concentrations have only increased from 0.035% to 0.045% (Ehleringer & Cerling 1995). While this increase has considerable ramifications for global climate, it is has minimal impact on the availability of CO_2 for carbon fixation in land plants. The atmosphere is mostly N_2 (78%) and O_2 (21%) (Mojzsis, 2001). This means less than 1 in 2,000 molecules diffusing into the leaf is a CO_2 molecule. Many commercial horticulture facilities augment CO_2 in greenhouses, but safety and monetary factors limit current enhancement strategies (Jeong, et al. 1993). CO_2 becomes unsafe at concentrations above 0.08%, concentrations above 0.2% should be avoided, and concentration of 5% or greater are fatal (Jeong, et al. 1993). Many greenhouses augment atmospheric concentrations to 0.15 to 0.2% despite these safety concerns. This is because up to a 30% increase in plant yield can result from this augmentation (Hicklenton and Jolliffe 1978, Yelle et al. 1990). Further complicating this augmentation strategy is that much more CO_2 is used to change the local atmosphere within the greenhouse than is actual taken up by plants, and much of the CO_2 added to the greenhouse atmosphere escapes to the outside (Jin et al 2009). Identifying an augmentation strategy that ensures safety and increases efficiency is desirable.

Previous research on CO_2 augmentation have consider the optimum concentration to be 800 ppm - 1000 ppm (Jin et al. 2009). The preferred delivery method for this has been to deliver pure CO_2 gas via diffusion in ventilated and unventilated greenhouses. Jin et al 2009 used a composting source of CO_2, where gas was generated by the decay of plant and livestock biomass.

Industry applies both of these methodologies and also a technique that produces CO_2 via natural gas generators.

Higher photosynthetic activity produces increased biomass as a result previous work has been focus on crop producing species, such as tomatoes, lettuce and celery. Biomass increases of up to 30% have been reporter on tomatoes with total fruit yield increase of 30% (Hicklenton and Jolliffe 1978, Yelle et al. 1990). Total yield increase varies with species and varieties, tomato varietals for example yield increases in biomass varying from 10% to 30%. This increase in biomass is also associated with a shorter overall growth period (Hicklenton and Jolliffe 1978).

Higher biomass, yield and shorter growth period are all of great interest, however it does not come without limitations. High atmospheric concentrations of CO_2 are dangerous to humans and some damage even shown in plants at concentrations higher than 3000 ppm (Kimball and Mitchell 1979). This has also been link nutrient availability and temperature where all factors for photosynthetic activity are critical for optimum environment. Nutrient uptake, in particular N_2, has been shown to be affected by temperature and further affected under CO_2 augmentation. (Jin et al 2009). The monetary concerns of the efficiency of CO_2 augmentation is a limitation that previous works have approach from enclosed systems to allow limited gas escape (Kimball and Mitchell 1979). Limiting CO_2 loss still faces a safety concern that has not been known to be address.

While atmospheric diffusion of gas into a plant's leaf superficially seems a simplistic physiological delivery mechanism, the leaf is, in fact, a complex organ system designed to efficiently carry out photosynthesis. From the cuticle, a protective layer which function is to prevent water loss to the mesophyll cells that allow CO_2 to move within the leaf. The spongy

mesophyll allows interchange of CO_2 and works in tandem with the palisade which contains chloroplast bearing cells that absorb light and execute photosynthesis. Stomata, are a pore like structure on the leaf's lower epidermis facilitating gas exchange. Carbon dioxide enters the stomata by gaseous diffusion for use in photosynthesis. The diffusion gradient also allows the photosynthetic byproduct, O_2, to exit the leaf. The epidermis which includes the stomata has several functions within the leaf; including protecting water loss, regulation of gas exchange and secretion of metabolic compound. This complex organ system cohesively and efficiently act as the primary site of photosynthesis.

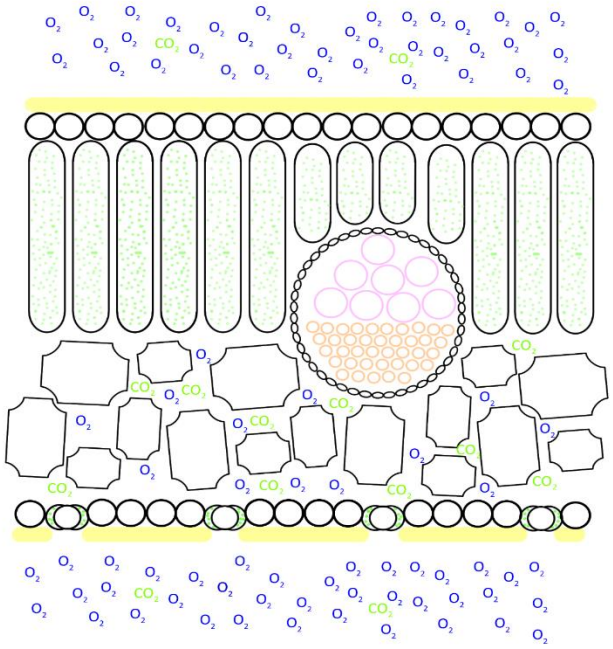

Figure 2. Leaf cross section, and gas exchange illustration.

Most of the energy humans' use is obtained from terrestrial plants, making techniques for increasing photosynthetic of interest to agricultural producers. CO2 GRO, Inc. is a corporation using a new technology for CO_2 delivery via foliar spray to increase plant yield. Previous

research on CO_2 enhancement to increase photosynthetic activity have only focused on terrestrial plants. Algae, an extremely evolutionarily diverse group of single cell organisms also rely on photosynthesis to produce carbohydrates, however contrary to terrestrial plants, they lack stomata and have a direct infusion of CO_2 (Julius, 2018). The hollow carbon fiber infusion of CO_2 on algae culture resulted on increase growth rate validating results of increase photosynthetic activity. This work adapts the algal based CO_2 enhancement techniques for terrestrial plants. CO2 GRO, Inc.'s technology delivers CO_2 via a foliar spray by creating a water holding film around each individual leaf. This film creates a microenvironment of high CO_2 concentration and allowing the diffusion gradient changes. In this research, we examine the use of CO_2 infused foliar spray as a mechanism to enhance CO_2 uptake by leaves and its availability as a photosynthetic resource.

Chapter II: Research Experiments

Observations of Initial Plant Responses to Foliar Misting

Initial experimentation was designed to identify the impacts of long term (germination to harvest) exposure CO2 enriched foliar spray. It was decided to test if short term physiological modification in plants could be observed in response to CO2 enriched foliar spray, while longer term experiments were underway. In an initial trial, romaine lettuce (the target species for the first experiment) was misted with CO2 enriched water every 15 minutes for a four hour period. During each 15 minute interval a 5 mm disc was cut from the lettuce leaf for chlorophyll a extraction. Each disc represented approximately 1 mg of plant material. Chlorophyll A was extracted using a 90% acetone solution and then quantified using standard methods with a Turner TD-700 Fluorimeter. Results of this initial experiment showed a 4 fold sustained increase in chlorophyll A over control cuttings from the first to final 15 minute misting interval.

The experiment was repeated a second time. However, this experimental replicate was run at 15 minute intervals for 2 hours. Chlorophyll A was measured using an Apogee MC-100 Chlorophyll Concentration meter. This meter allowed chlorophyll A to be estimated without cutting the leaf or damaging the plant in any other fashion. Chlorophyll A is reported as a unit area rather than extraction by weight and the meter estimates chlorophyll A directly by contact on the leaf's surface. Results from the second experiment were consistent with the first. A statistically significant (p=0.010477, t test) increase (~30%) in chlorophyll A per m2 of leaf surface area was observed over the duration of the experiment beginning with the first 15 min interval.

Notable in these initial experiments is the rapidity of physiological response seen in CO2 exposed plants. This data is encouraging and consistent with the hypothesis of significant growth enhancement with CO2 delivery via foliar spray**.**

Table 1

Fifteen-minute measurements of chlorophyll A post CO2 enhanced misting event.

Time Interval (min)	Treatment	Control
15	8.5	8.3
30	13.1	8.3
45	13.1	8
60	11	8.6
75	9.1	8.3
90	10.3	8.5
105	13.1	8
120	12.2	8.3

Replication of Instantaneous Responses with Stomatal Activity Observed

The initial experimentation was repeated and observations of stomatal conductance were added. This metric estimates the amount of gas exchange and water vapor loss occurring via the stomata. A porometer (ICT International SC-1) is the specific instrument used to measure this parameter. Three experiments were run with the porometer. In all experiments chlorophyll A concentration were measured (Apogee MC-100). In the first, both metrics were quantified every 20 minutes for 100 minutes. Two treatments were consider: 1) CO2 enriched foliar spray and 2) no spray. Data for each metric was compared between treatments using a tTest for equal means. Both chlorophyll A (p=0.0077) and stomatal conductance (p=0.0131) showed significant increases in the CO2 exposed treatments.

The second and third experiment were identical in treatments and metrics quantified. They only differed in duration of the experiment. This is a result of the time it takes to acquire a stomatal conductance estimate in comparison to chlorophyll A. In the second experiment the duration was 2 hours and 20 minutes and the third lasted 4 hours. Treatments for these experiments included: 1) CO2 enriched foliar spray, 2) unenriched foliar spray, and 3) no spray. The unenriched foliar spray treatment was added to potentially reject the hypothesis that water vapor alone could explain the results from prior experiments. In both experiments chlorophyll A was measured for 5 randomly selected leaves every 10 minutes immediately following treatments which were also applied every 10 minutes. Stomatal conductance was measured each hour for each treatment. Both experiments were consistent in showing higher chlorophyll A content and higher stomatal conductance in CO2 exposed treatments. ANOVA was used to compare chlorophyll A data in both experiments and stomatal conductance in the third experiment (only two estimates existed for experiment 2 making statistical comparison impossible). Significant differences existed between CO2 exposed treatments for chlorophyll A (p=0.00057, exp2 and p=0.0000005.5, exp3) and stomatal conductance (p=0.00000074). Notable is that NO significant difference existed between unenriched spray and no spray treatments, strongly suggesting that CO2 availability was the factor increasing both chlorophyll A and stomatal conductance.

Important is that after 5 rounds of instantaneous experimentation, chlorophyll A content consistently shows a 10-20% increase in plants exposed to CO2 enriched foliar compared to plants receiving no foliar spray or non-augmented foliar spray. Further, 3 rounds of experimentation show a consistent increase in stomatal conductance of > 8 times in plants

exposed to CO2 enriched foliar compared to plants receiving no foliar spray or non-augmented foliar spray. This data continues to be encouraging and consistent with the hypothesis of significant growth enhancement with CO2 delivery via foliar spray.

Table 2

Chlorophyll A content measured in 15 minute intervals for second instantaneous response experiments.

Treatment 1 (CO_2 Enriched Foliar Spray)					Treatment 2 (Unenriched Foliar Spray)					Treatment 3 (No Spray)				
Leaf 1	Leaf 2	Leaf 3	Leaf 4	Leaf 5	Leaf 1	Leaf 2	Leaf 3	Leaf 4	Leaf 5	Leaf 1	Leaf 2	Leaf 3	Leaf 4	Leaf 5
4.8	7.8	6.1	5	5	5.8	4.8	7	5.6	5.2	6	4.1	6.6	3.8	6.4
7.1	4.9	4.7	6.3	7.3	5.6	6	5.8	4.3	5.1	4.2	6.2	6.4	7	4.1
4.1	5.9	4.9	5.2	4.4	4.3	6.4	5.1	4.1	4.2	6.1	6.7	6.7	4.4	3.7
5.7	7.3	4.5	5.2	4.5	6.3	6.1	4.4	5.5	4.5	5.3	4	6.7	5.2	5.4
4	4.8	7.1	5.5	6.4	5.6	5.4	5.4	5.2	5.6	6.4	4.2	7.9	4.1	6.1
4.9	6.1	6.7	5.4	5.5	5.5	4.6	5.8	4.2	5.3	4.8	3.7	3.8	4.6	5.3
6.7	5.5	3.9	5.8	5.4	4.2	6.1	5	6.4	3.9	6.5	6.9	3.9	6.1	3.8
6.6	7.2	4	6.2	5.7	4.4	5	5.8	4	5.5	3.8	6	4.3	5.3	6.2
5.5	7.2	6.1	4.9	5.3	5.4	5.3	5	6.1	4.1	6.9	4	5.9	3.6	3.3
4.8	5.2	5.5	5.5	6.3	5.2	5.1	5.4	4.3	3.8	4.1	4.4	5.4	4.6	3.8
5.1	6.1	6.5	4.8	4.5	4.9	6.3	5.6	5.4	4.2	5.8	4.1	3.5	6.9	5.7
6.5	6.4	5.7	5.4	5.6	5	6.2	4.9	5.9	5.3	5.3	6.7	4.1	5.7	4.1
4.4	6.3	5.6	4.1	5.4	5.2	5.2	4.8	6.1	4.8	4.1	4.2	5.4	5.5	6.9
5.6	5.2	4.6	5.9	5.5	4.7	4.9	3.7	5.4	3.6	6.4	5.5	4.2	3.8	4.1
4.3	5	6.8	6.5	4.4	5.4	4.9	6.9	4.5	4.3	5.9	6.5	4.5	3.3	3.8
4.9	5.3	5.8	4.8	6.9	4.6	4.7	4.7	4.9	4	3.9	6.4	5.9	6.3	3.6
6.3	6.4	6.1	4.9	4.8	5.1	4.8	6.6	5.1	4.4	3.6	3.2	6.4	6	4.6
6.4	6.3	5.7	4.5	5.3	4.6	5	4.2	5.6	4.5	3.6	7.2	5.8	6.3	4.9
4	5.7	5.9	4.2	6.8	4.9	3.7	4.1	5.5	5.3	4.1	6.8	5.6	5.3	5.1
5.3	5.6	5.7	4.7	4.9	4.5	4.9	4.6	6	4.1	4.1	4.2	5.6	3.6	6.6
5.7	5.5	4.3	6.6	5.5	3.9	5.1	4	4.6	5.1	3.1	5.7	4.2	6.9	6.5
4.1	6.9	6	4.6	5.5	6.6	5.3	4.9	5	5.2	4.1	3.5	5.7	6.6	5.1
5.8	5.2	4.9	5.2	3.9	4.2	4.7	5	5.4	4.8	3.6	5.7	4.2	4.2	6
5.6	5.8	6.6	4.9	4.7	5.1	4.5	6.3	5.7	4.5	5.8	6.4	4.6	3.2	6.1
5.8	5.8	4.6	4.3	5.8	5.1	3.9	4.6	5.4	4.3	3.5	6.6	6	3.6	3.2

Table 3

Hourly porometer data for second instantaneous response experiments.

	Stomatal Conductance		
	Treatment 1 (CO_2 Enriched Foliar Spray)	Treatment 2 (Unenriched Foliar Spray)	Treatment 3 (No Spray)
	705.1	128.5	257.9
	1036.7	94.4	185.8
	865.9	103.9	205.9
	1160.6	49.1	98.9
Average	942.075	93.975	187.125

Observations of Instantaneous when Stomata are Blocked

In our previous experiments, data for instantaneous physiological responses in plants treated with CO_2 enriched foliar spray were observed. These prior experiments found rapid increases in chlorophyll A and stomatal conductance in response to exposure to foliar spray with CO_2 infused water. Values for stomatal conductance were an order of magnitude greater in foliar sprayed leaves in comparison to control leaves. These results suggested that CO_2 may be entering the leaf independent of the stomata and two experiments were proposed to test the validity of this hypothesis.

In these experiments the leaf's upper surface was treated instead of the bottom side. Stomata are typically scarce on the leaf's upper side and abundant on the bottom. In each experiment two treatments were considered. 1) Control – no foliar spray and 2) CO_2 enriched foliar spray. The sprayed leaves were exposed every 10 minutes and control and sprayed leaves were measured for stomatal conductance (ICT International SC-1). Treatment and measurement continued for 180 minutes. The two experiments varied in how the leaf's bottom was prepared.

In the first experiment no modification was made to the leaf bottom and in the second experiment the leaf's bottom was covered with petroleum jelly to prevent stomatal conductance.

Both experiments were consistent in showing higher stomatal conductance in CO_2 exposed treatments. A T-test was used to compare stomatal conductance data. Significant differences existed between CO_2 exposed treatments in both experiments (exp 1: $p=1.369X10^{-9}$, exp2: $p=2.743x10^{-11}$).

Results from these experiments suggest that the CO_2 rich microenvironment surrounding the leaf created by foliar spray is capable of bypassing the leaf's cuticle. The cuticle is a waxy covering that has evolved to prevent water loss in the plant. It appears CO_2 can move through cracks in the cuticle and then cross the cells epidermal membrane through standard cellular transport processes.

Table 4

Stomatal conductance in plants with unobstructed stomata.

Control	CO2-Foliar Spray
48.1	59
36.4	241.8
83.3	913.8
62.6	1351.3
66.1	986.2
130	1111.5
72	595.2
84.9	1250.1
35	441.6
56.7	1240.3
66.5	852.4
52.6	730.6
36.8	849.6
32.8	649.1
35.7	1030.4
43.3	433.8
78.8	1120.8

Table 5

Stomatal conductance in plants where stomata were blocked with petroleum jelly.

Petroleum Jelly	
Control	CO2-Foliar Spray
33.3	59
29.6	1499.8
38.7	2077.1
42.7	1105.6
49.1	1115.1
37.7	975.2
41.2	1075.9
46.1	1310.9
50	1516
84.6	1071.8
28.9	1128.4
37.8	932.2
47.7	807.5
50.3	1400
35.1	1037.9
38.5	875.5

Conclusions Bases Instantaneous Response Experimentation

The increases in Chlorophyll A are consistent with additional carbon dioxide being available for the plant for assimilation. This is consistent with increased stomatal conductance activity which suggests greater levels of gas exchange are occurring in plants misted with CO_2 saturated water compared with plants misted with unsaturated water and non-misted plants. The stomata conductance activity was above the capacity of plant stomata. Blocking stomata had no effect on gas exchange activity. This suggests that foliar misting of CO_2 saturated water allow gas exchange to occur independently of stomata. This is likely driven by a strong diffusion gradient created by gas saturated water allowing trans cuticular transport of gasses.

Chapter III: Commercial Growth Trial Data

CO2 GRO installed a CO_2 spray infusion system on The Growcer's (https://www.thegrowcer.ca/growing-systems) demonstration containerized grow system (CGS) housed at their corporate headquarters in Ottawa, Canada. A kale (*Brassica oleracea*) varietal was grown for a 10-week period (Black Magic Kale https://www.johnnyseeds.com/vegetables/kale/black-magic-kale-seed-3531.html#q=dinosaur%2Bkale&lang=en_US&start=1). The experiment began on 12-April-19 and ended 21-June-19. Control (untreated) and CO2 infused spray treated plants were grown simultaneously in the CGS. Each treatment comprised 11 individuals. Treated plants were sprayed every 15 minutes for 10 seconds with a mist enriched with CO_2 to 80-100% saturation. At 10 weeks three randomly selected leaves were measured for chlorophyll A per unit area with a SPAD based meter. These values were used to obtain an average for each plant. Once chlorophyll A measurements were completed plants were obtained, plants were harvested and dried at 60°C for 24 hours. Dried plants were then weighed to obtain a biomass estimate for each individual. Chlorophyll A and biomass measurements were compared using a 1-sided T-test. For each parameter a statistically significant difference was found between the control and treated plants, with the treated plants having both a higher chlorophyll A content and higher biomass.

Table 6

Chlorophyll A measurements for experimental plants at harvest.

	Chlorophyll A Data (SPAD Unit)	
Replicate #	Control	CO2 Spray
1	74.9	81.9
2	25.5	55.2
3	53.9	73.4
4	66.9	56.6
5	41.2	34.1
6	53.3	69.6
7	4.3	32.5
8	70.1	57.1
9	41	104
10	38.7	65.6
11	48	67.8
Average	47.0727	63.4363

Table 7

Dry weight measurements for plants at harvest.

	Dry Weight Data (kg)	
Replicate #	Control	CO2 Spray
1	0.018	0.032
2	0.012	0.045
3	0.022	0.078
4	0.048	0.049
5	0.032	0.044
6	0.058	0.064
7	0.066	0.046
8	0.054	0.056
9	0.052	0.072
10	0.016	0.032
11	0.028	0.042
Average	0.0369	0.0509

Significant differences were identified in both average Chlorophyll A (p= 0.0374) and dried wet (p= 0.03579) between treatment and control groups based upon t test analysis of data. Dry weight was ~38% greater on average in the treatment group and Chlorophyll A was ~35% greater in the treatment group. This is consistent with the foliar spray with CO2 infused water delivering CO_2 to plant for photosynthesis at rates above the ambient atmosphere.

Pathogen Based Experimentation

CO2GRO Inc's gas infusion system has been documented to enhance plant growth significantly in comparison to other commercial CO_2 enhancement methods. Unexpected during these growth trails, was a reduction in bacterial, fungal, and insect loads preying on plants treated with CO_2 infused water. This observation is of great interest and was identified as a high priority for validation. This series of experimentation was established to validate observations in pathogen and prey reduction.

Three pathogenic organisms were consider in this experimentation; *Escherichia coli* (Bacteria), *Leveillula taurica* (Powdery Mildew), and *Fusarium oxysporum* (Fungi). Experimental design and results are reported by pathogen below.

***Escheirichia coli* Results**

Two experiment were conducted. In the first, ten replicate nutrient plates were maintained for 5 days. All plates were inoculated with *E. coli* via streaking from a stock culture. Five plates were allowed to grow for a 5 day duration without treatment. The remaining five plates were treated with CO_2 infused water (800ppm) via a spray bottle. Plates were sprayed

daily every 15 minutes for a 3 hour interval. Bacterial count estimations were conducted each day post inoculation of the 5 day experiment.

Table 8

Bacterial colony count estimations on petri dishes inoculated with E. coli. Treatment plates (T1 T5) were misted with CO2 saturated foliar spray and control plates were allowed to grow without modification.

	Day 1	Day 2	Day 3	Day 4	Day 5
T1	Inoculation	10	10	100	100
T2	Inoculation	10	10	100	100
T3	Inoculation	10	10	100	100
T4	Inoculation	10	10	100	100
T5	Inoculation	10	10	100	100
C1	Inoculation	100	1000	100000	1000000
C2	Inoculation	100	1000	100000	1000000
C3	Inoculation	100	1000	100000	1000000
C4	Inoculation	100	1000	100000	1000000
C5	Inoculation	100	1000	100000	1000000

In the second experiment, plant leaves were used in the place of nutrient agar plates. Twenty leaves on four plants were infected with *E. coli*. Five leaves were allowed to grow for a 5 day duration without treatment. The remaining five leaves were treated with CO_2 infused water (800ppm) via a spray bottle. Leaves were sprayed daily every 15 minutes for a 3 hour interval. Bacterial count estimations were conducted on days 3 and 5 using an agar paddle plate inoculated with plant leaves.

Table 9

Bacterial counts on randomly selected plant leaf post inoculation with E. coli.

	Day 1	Day 3	Day 5
T1	Inoculation	0	10
T2	Inoculation	0	100
T3	Inoculation	0	10
T4	Inoculation	0	0
T5	Inoculation	0	100
C1	Inoculation	100	10000
C2	Inoculation	100	10000
C3	Inoculation	100	10000
C4	Inoculation	100	10000
C5	Inoculation	100	10000

In both experiments, results were compared statistically with a Mann Whitney U test for equal medians using Day 5 data. This non-parametric test was selected because bacterial abundances were categorical rather than ordinal. Statistical comparisons found significant differences in both experiments (p=0.003978 and p=0.0070887). These results are consistent with the hypothesis that CO_2 infused foliar spray inhibits the growth of *E. coli*. (Data file submitted with this report.)

Fusarium oxysporum **Results**

Two experiment were conducted. In the first, ten replicate potato agar plates were maintained for 5 days. All plates were inoculated with *F. oxysporum* via streaking from a stock culture. Five plates were allowed to grow for a 5 day duration without treatment. The remaining five plates were treated with CO_2 infused water (800ppm) via a spray bottle. Plates were sprayed daily every 15 minutes for a 3 hour interval. Fungal colony forming unit (CFU) estimations were conducted each day post inoculation of the 5 day experiment.

Table 10

Fusarium oxysporum colony count estimations on petri dishes. Treatment plates (T1-T5) were misted with CO2 saturated foliar spray and control plates were allowed to grow without modification.

	Day 1	Day 2	Day 3	Day 4	Day 5
T1	Inoculation	0	0	0	10
T2	Inoculation	0	0	0	0
T3	Inoculation	0	0	10	100
T4	Inoculation	0	0	10	10
T5	Inoculation	0	0	0	0
C1	Inoculation	0	10	1000	10000
C2	Inoculation	10	1000	10000	100000
C3	Inoculation	10	1000	10000	100000
C4	Inoculation	0	10	1000	10000
C5	Inoculation	10	1000	10000	100000

In the second experiment, plant leaves were used in the place of potato agar plates. Twenty leaves on four pepper plants were infected with *F. oxysporum*. Five leaves were allowed to grow for a 5 day duration without treatment. The remaining five leaves were treated with CO_2 infused water (800ppm) via a spray bottle. Leaves were sprayed daily every 15 minutes for a 3 hour interval. Fungal CFU estimations were conducted on days 3 and 5 using an agar paddle plate inoculated with plant leaves.

In both experiments, results were compared statistically with a Mann Whitney U test for equal medians using Day 5 data. This non-parametric test was selected because bacterial abundances were categorical rather than ordinal. Statistical comparisons found significant differences in both experiments (p=0.010418 and p=0.009937). These results are consistent with the hypothesis that CO_2 infused foliar spray inhibits the growth of the fungus *F. oxysporum*.

Table 11

Fusarium oxysporum counts on randomly selected plant leaf post inoculation with the fungus.

	Day 1	Day 3	Day 5
T1	Inoculation	0	10
T2	Inoculation	0	10
T3	Inoculation	0	10
T4	Inoculation	0	0
T5	Inoculation	0	100
C1	Inoculation	100	10000
C2	Inoculation	100	10000
C3	Inoculation	10	1000
C4	Inoculation	10	1000
C5	Inoculation	10	10000

Leveillula taurica **Results**

A single long term experiment was conducted with the Powdery Mildew, *Leveillula taurica.* The organism is an obligate parasite preventing experimentation with plate agar cultures. The experiment consisted of three treatments using 8 replicate pepper plants. The treatments consisted of an untreated control, plants grown in a CO_2 enriched atmosphere (1200 ppm), and plants treated with CO_2 infused water (800ppm). All plants were exposed to *Leveillula taurica* collected from wild plants. The inoculation procedure was as follows. Collected leaves were dried and ground. Ground leaves were infused in water for 24 hours and then filtered. Filtered water was spray on test plants to complete fungal infection. Plants were allowed to grow for 21 days. The first signs of fungal infection were recorded for each test group and the number of days survived were recorded for each plant.

Results were compared statistically using an ANOVA and Tukey's Post-Hoc test using the "Days Survived" data. The ANOVA identified a significant difference among experimental

treatments (p=1.018E-9). Further analysis with the Tukey's Post-Hoc test identified the significant differences occurred between CO_2 infused foliar spray treated plants and all other treatments. No significant differences were identified between the control plants and plants grown in CO_2 enriched atmosphere. These results are consistent with the hypothesis that CO_2 infused foliar spray treatment inhibits the growth of Powdery Mildew, *Leveillula taurica.*

Table 12

Survival data for plants inoculated with Powdery Mildew, Leveillula taurica under multiple growth environments.

Control	Days Survived	CO2 Gas	Days Survived	Foliar Spray	Days Survived
C1	6	G1	7	S1	14
C2	6	G2	8	S2	21
C3	7	G3	8	S3	21
C4	8	G4	9	S4	21
C5	9	G5	9	S5	21
C6	9	G6	9	S6	21
C7	9	G7	10	S7	21
C8	15	G8	11	S8	21

Chapter IV: Conclusion

This experimentation suggests CO_2 infused foliar misting is an effective technique for supplementing the carbon requirements for plants for assimilation during photosynthesis. Experimental plants responded quickly when exposed to CO_2 via foliar misting. Results indicated that bulk CO_2 transfer occurred independently of the stomata. This is likely the effect of a diffusion gradient created by the aqueous microfilm created during misting. The differential between gasses within the aqueous microenvironment and the leaf's interior force CO_2 into the leaf and other gases out of the leaf. Responses in plant growth equivalent to atmospheric augmentation with CO_2 were obtained in a commercial growth setting. Important to this was the observation and documentation of pathogen suppression caused by the fluctuating pH in the aqueous microfilm. The ability to suppress pathogens may have application independent of CO_2 augmentation, but a common practice by commercial growers is to avoid watering leaves in favor of soil watering. Water on the leaf's surface encourages pathogenic organism growth typically. The pH suppression of these pathogens alleviates these concerns and make the foliar misting technique more viable for commercial environments. Advantages of this technique over other include efficiency and safety. Far less CO_2 is used with the foliar misting technique than with other commercial CO_2 enhancement technologies and all gas is contained within the aqueous microfilm surrounding the plant's leaf. Other CO_2 deliver techniques require the atmosphere to be elevated to 800ppm or greater which is harmful to human health. This reduction and containment of CO_2 maintains human health, decreases costs, and limits environmental output of CO_2.

References

Balegh, S. E and Biddulph, O. 1970. "The photosynthetic action spectrum of the bean plant". *Plant Physiology.* 46 (1): 1–5.

Bassham, J. A., Calvin, M., & Benson, A. A. (1950). The Path of Carbon in Photosynthesis: The Role of Malic Acid. University of California Radiation Laboratory.

Berg JM, Tymoczko JL, and Stryer L. 2002. *Biochemistry*. 5th edition. New York: W H Freeman; 2002.

Bryant, DA and Frigaard, NU, 2006. Prokaryotic photosynthesis and phototrophy illuminated, *Trends in Microbiology*, 14 (11): 488–496.

Ehleringer, J. R., & Cerling, T. E. (1995). Atmospheric CO2 and the ratio of intercellular to ambient CO2 concentrations in plants. Tree physiology, 15(2), 105-111.

Farquhar GD, von Caemmerer S, and Berry J. 1980. A biochemical model of photosynthetic CO2 assimilation in leaves of C3 species. *Planta* 149: 78–90

Hicklenton, P. R., & Jolliffe, P. A. (1978). Effects of greenhouse CO2 enrichment on the yield and photosynthetic physiology of tomato plants. Canadian Journal of Plant Science, 58(3), 801-817.

Jeong, B. K., Fujiwara, K., & Kozai, T. (1993). Carbon dioxide enrichment in autotrophic micropropagation: Methods and advantages. HortTechnology, 3(3), 332-334.

Jin, C., Du, S., Wang, Y., Condon, J., Lin, X., & Zhang, Y. (2009). Carbon dioxide enrichment by composting in greenhouses and its effect on vegetable production. Journal of Plant Nutrition and Soil Science, 172(3), 418-424.

Julius M. 2018. Chapter 6: Carbohydrate Diversity in Microalgae: A Phylogenetically Arranged Presentation.pp. 133-144. *IN*: Levine, I. A., & Fleurence, J. *Microalgae in Health and Disease Prevention. Academic Press*. N.Y.

Kimball, B. A., & Mitchell, S. T. (1979). Tomato yields from CO2-enrichment in unventilated and conventionally ventilated greenhouses.

Kirschbaum M. 2011. Does Enhanced Photosynthesis Enhance Growth? Lessons Learned from CO2 Enrichment Studies. *Plant Physiology*. 155 (1): 117-124.

Mojzsis, S. J. (2001). Life and the Evolution of Earth's Atmosphere. Earth inside and out, 32-39.

Ort DR, Merchant SS, Alric J, Barkan A, Blankenship RE, Bock R, Croce R, Hanson MR, Hibberd JM, Long SP, Moore TA, Moroney J, Niyogi KK, Parry MA, Peralta-Yahya PP, Prince RC, Redding KE, Spalding MH, van Wijk KJ, Vermaas WF, von Caemmerer S, Weber AP, Yeates TO, Yuan JS, and Zhu XG. 2015. Redesigning photosynthesis to sustainably meet global food and bioenergy demand. *Proceedings of the National Academy of Sciences of the United States of America, 112*(28): 8529–8536.

Raven PH, Evert RF, and Eichhorn SE, 2005. *Biology of Plants* (7th ed.). New York: W. H. Freeman and Company. pp. 124–127. ISBN 978-0-7167-1007-3.

Tewolde F. T., Shiina K., Maruo T., Takagaki M., Kozai T., and Yamori W. 2018. Supplemental LED inter-lighting compensates for a shortage of light for plant growth and yield under the lack of sunshine. *PloS one*, *13*(11): e0206592.

Yelle, S., Beeson, R. C., Trudel, M. J., & Gosselin, A. (1990). Duration of CO2 enrichment influences growth, yield, and gas exchange of two tomato species. Journal of the American Society for Horticultural Science, 115(1), 52-57.