

# Research Report #1

## St. Cloud State University

CO2 GRO Inc. has engaged St. Cloud State University (SCSU) and in particular Dr. Matthew Julius and his research team to evaluate the veracity of CO2 GRO's CO2 Foliar Spray Technology, its effect on plant growth and plant physiology. SCSU is conducting a series of four experiments to document the impacts of CO<sub>2</sub> delivered in supersaturated water to stomata via foliar misting. The first of these experiments has been initiated with seeding plants being prepared for foliar spray exposure or other control/ null treatments. Ongoing data from this experiment will be reported in future reports.

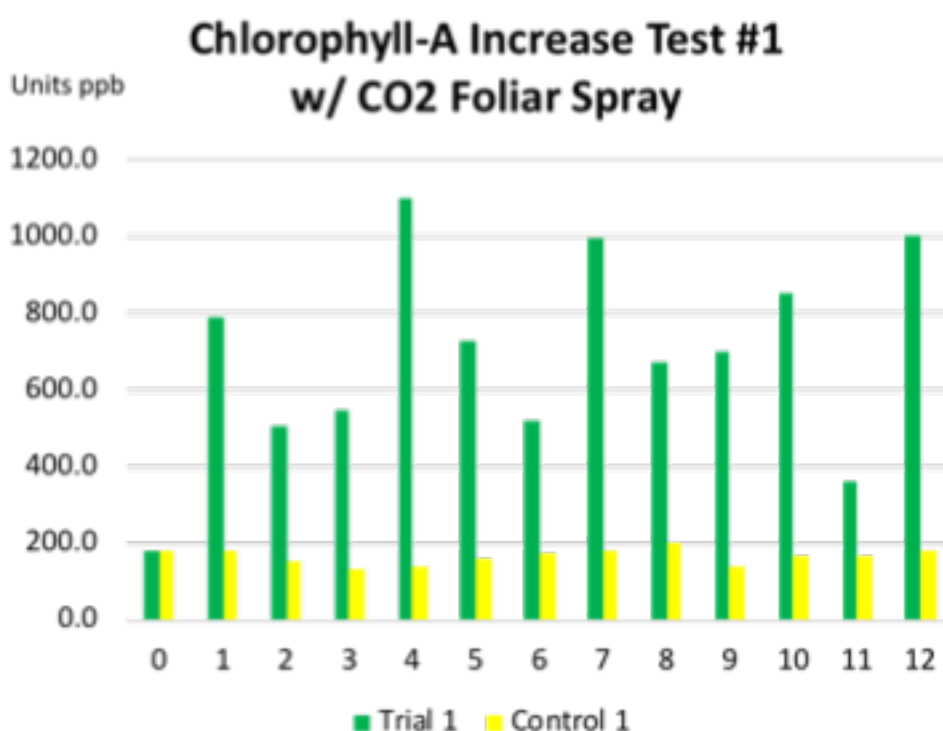
Initial experimentation was designed to identify the impacts of long term (germination to harvest) exposure CO<sub>2</sub> enriched foliar spray. It was decided to test if short term physiological modification in plants could be observed in response to CO<sub>2</sub> enriched foliar spray, while longer term experiments were underway. In an initial trial, romaine lettuce (the target species for the first experiment) was misted with CO<sub>2</sub> 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.

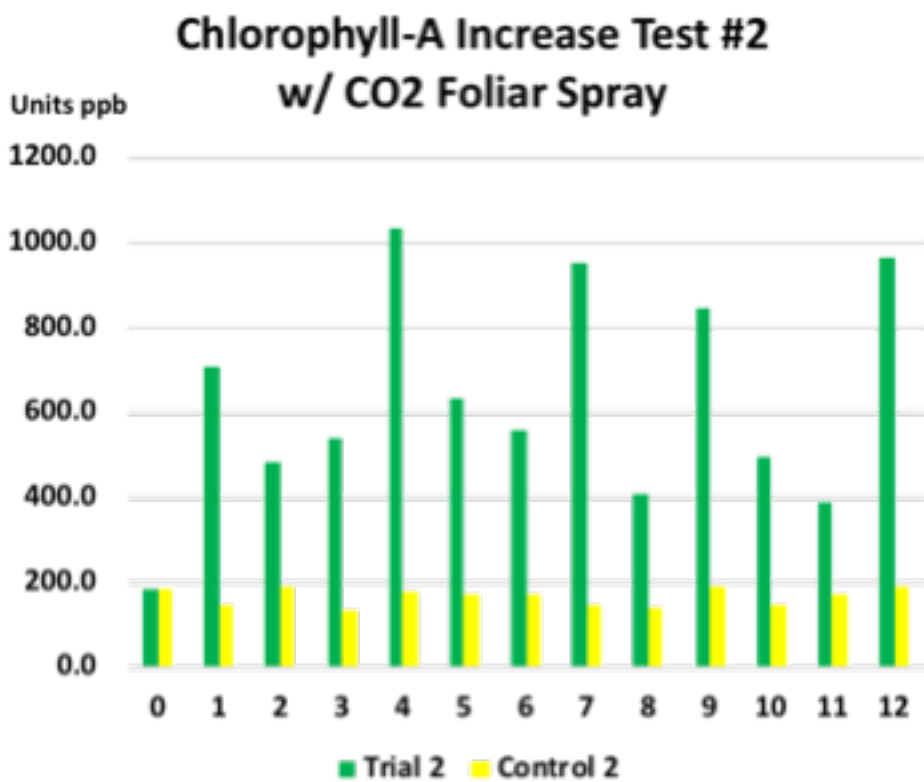
The experiment was repeated a second time. However, this experimental replicate was run at 15 minute intervals for 2 hours. Chlorophyll A was measured using an Apogee MC-100 Chlorophyll Concentration meter.

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 ( $p=0.010477$ , test) increase (~35%) in chlorophyll A per m<sup>2</sup> of leaf surface area was observed over the duration of the experiment beginning with the first 15 min interval.

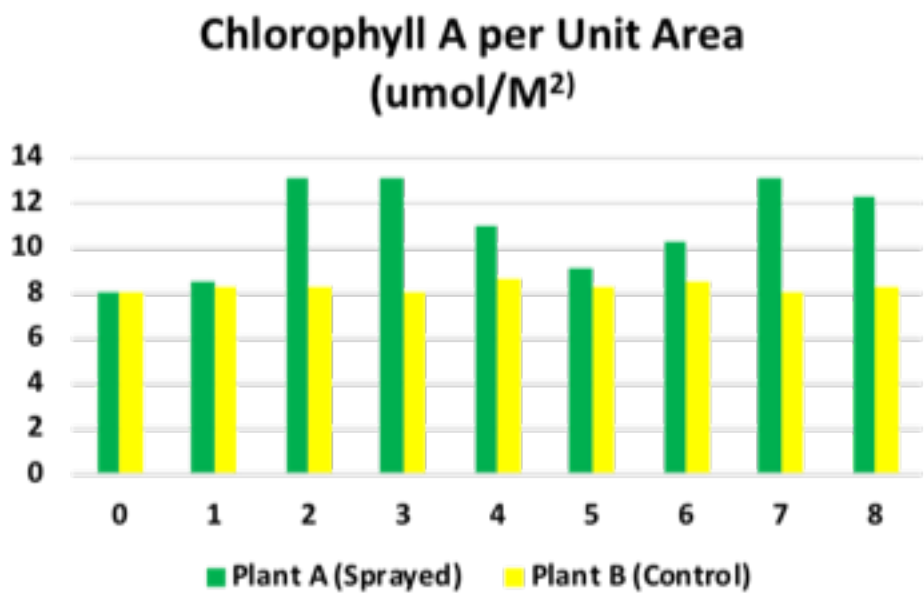
This experiment is planned for replication a third time, with the addition of leaf porometer measurements. A SC-1 Leaf Porometer (ICT International) has been ordered for this experiment. This instrument will allow stomatal conductance of gasses to be measured in real time intervals in response to CO<sub>2</sub> enriched foliar spray.

Notable in these initial experiments is the rapidity of physiological response seen in CO<sub>2</sub> exposed plants. This data is encouraging and consistent with the hypothesis of significant growth enhancement with CO<sub>2</sub> delivery via foliar spray.





In the chlorophyll-A trials leaves were misted every 15 minutes for 3 hours. Two 5 mm circles are cut from the leaf. The Chlorophyll-A is extracted via acetone and then quantified spectrophotometrically.



In the chlorophyll-A units per area measurements, the leaves were misted with CO2 foliar spray every 15 minutes for two hours.

Conclusions. Results of the initial two experiments showed a 4 fold sustained increase in chlorophyll A over control cuttings from the first to final 15 minute misting interval. A statistically significant ( $p=0.010477$ , test) increase ( $\sim 35\%$ ) in chlorophyll A per  $m^2$  of leaf surface area was observed over the duration of the experiment beginning with the first 15 min interval. These results mirror all of the growth increase results that CO2 GRO Inc. has experienced to date with its novel PCT patent pending CO2 Foliar Spray technology.