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**The Effects of Elevated CO₂ Levels on Broad Bean, *Vicia faba*,
Growth/Defense Tradeoffs**

An Honors Thesis submitted in partial fulfillment of the requirements for Honors in the
Department of Biology.

By
Harley B. Kitching

Under the mentorship of *Dr. Edward B. Mondor*

ABSTRACT

Atmospheric changes, associated with global climate change, are increasing at an unprecedented rate. Plants generally display higher rates of growth in response to elevated CO₂ levels, but this response varies among species. In addition, very little is known about how plant growth/defense tradeoffs will be altered by increasing CO₂ levels. By raising Broad bean, *Vicia faba* L., plants under ambient (400 ppm) and elevated (900 ppm) levels of CO₂, it was shown that atmospheric composition directly altered plant growth/defense tradeoffs. Plants grown under elevated CO₂ had lighter stem weights but greater numbers of extrafloral nectaries and higher rates of extrafloral nectar secretion. Thus, plants grown under elevated CO₂ invested more in defense (extrafloral nectaries and extrafloral nectar production) than growth (biomass). These results indicate that CO₂ may act as a stressor for Broad bean plants.

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Introduction

Climate change has become a hot topic of discussion for journalists and politicians. Normally, when people reflect on climate change they think of polar bears or deglaciation (Shakun et al. 2012). There are many other pertinent issues that may come with climate change that are seldom discussed like floods, droughts, fires, disease outbreaks, risks to human health, and ecosystem destruction (Singh et al. 2016).

Most scientists believe that climate change is due to an increase of greenhouse gases like carbon dioxide (CO₂) in the atmosphere, due to human industrialization (Stiling et al. 2013). Since it has been determined that CO₂ is a primary greenhouse gas responsible for climate change, many studies have supported this claim (Konovalov et al. 2016). As CO₂ levels have increased by 40% since the industrial revolution (Konovalov et al. 2016), with 25% of these emissions coming from agricultural practices alone (Kontopoulou et al. 2014), scientists have started to ask how this greenhouse gas may alter ecological processes.

There has been widespread support for the concept that plants benefit from elevated CO₂ levels, as evidenced by increased above- and below-ground biomasses as well increased leaf sizes and numbers (Gray & Brady 2016). As plants flourish in an elevated CO₂ environment, it can be assumed that other species, especially herbivores, would also benefit due to the increased success of plants. The results may not be all positive, however. While plants exhibit increased growth in response to elevated CO₂ levels, nitrogen concentrations and overall plant quality is often negatively affected (Stiling et al. 2013). This situation could be even worse for wild plants, that are already nitrogen-limited (Kontopoulou et al. 2014). Many studies also fail to consider that other

factors such as rainfall or extreme heat waves might occur as climates are altered, that could further harm plant growth (Teixeira et al. 2011).

Plants must rapidly adapt to stressors in a changing environment in order to survive (Gray & Brady 2016). One plant species that can be used as a model organism to better understand the effect of changing atmospheric composition on growth is Broad bean, *Vicia faba*. Broad beans are nitrogen fixers, used during crop rotations, making them an interesting subject for biomass studies (Köpke & Nemecek 2009). It has previously been shown that *V. faba* seedling emergence and plant growth is altered once CO₂ has permeated the soil (Al-Traboulsi et al. 2012). In addition, *V. faba* stomata remain closed for extended periods of time when exposed to high levels of CO₂, which could be a defense mechanism for the plant to maintain homeostasis (Talbot et al. 1996).

Plants must defend themselves to survive and any allocation of resources to defensive actions usually comes at the expense of plant growth and development, as plant resources are finite (Huot et al. 2014). Defense in Broad beans is centered around small glands located near the petiole of leaves called extrafloral nectaries (EFNs). Many plants have EFNs, though they differ widely in the structures and mechanisms for excreting the nectar (Avalos et al. 2016). It is believed that EFNs evolved to attract beneficial insects such as ants that protect the plant from herbivores (Khazaei et al. 2014). Extrafloral nectary secretions are rich in carbohydrates which contributes to their ability to attract insects to defend the plant (Lüttge 2013). The sugars in these nectaries are thought to come from the phloem of the plant making them energetically costly to produce (Lüttge 2013). If a plant experiences a high volume of stress (e.g., increased herbivory), the amount of nectar produced from EFNs usually increases (Huot et al. 2014).

In this study, the effects of elevated CO₂ atmospheres on Broad bean, *Vicia faba*, growth and defense were assessed. Growth was directly evaluated by measuring traits such as numbers of leaves and root/shoot weights. Defenses were assessed by counting the number of EFNs and measuring rates of extrafloral nectar production.

Methods

AtmoSim 2100

The AtmoSim 2100 is a modified growth chamber designed and built in collaboration with Spencer Harp, a laboratory supervisor in Georgia Southern University's Mechanical Engineering Department. The AtmoSim 2100 consists of eight, 20-gallon aquaria arranged in two rows using a metal frame to create four upper-level and four lower-level chambers. Aquaria are accessed through a clear Plexiglas sheet covering the tops of the chambers. A fuzzy logic, computer-controlled Sentinel Analyzer regulates the CO₂ levels, from a large CO₂ tank, that is allowed to flow into each chamber. The Sentinel Analyzer (Model CHHC-4, Sentinel Global Products Solutions Inc., Santa Rosa, CA 95403) also monitors other abiotic factors such as temperature and humidity. Above each group of four chambers are two 125-watt Hydrofarm (Hydrofarm, Medley, FL 33178) grow lights. Before the initial trial, the AtmoSim 2100 was calibrated to ensure accurate CO₂ concentrations in each chamber. The upper-level chambers were set at 400 ppm CO₂ (ambient) while the lower-level chambers were set at 900 ppm CO₂ (elevated) (Figure 1). Temperature, humidity, and CO₂ levels were all monitored using the Sentinel Analyzers. The minimum and maximum reading for all three variables were recorded weekly, throughout the duration of this experiment.

Experimental Design

For each of two trials, two Broad bean, *Vicia faba*, plants were grown in each chamber for a total of sixteen plants. One seed was placed in each pot, and one pot was placed in the front and the other in the back of each chamber. The plants were watered

every other day. Plant traits were assessed three times. The first assessment occurred on the second week of growth for trial one and on the third week of growth for trial two. The second assessment was conducted three days after the first measurements. The final assessment was taken after three weeks of growth for trial one and after four weeks of growth for trial two.

For each assessment, height (cm), numbers of immature leaves, number of fully developed leaves, number of extrafloral nectaries, and amount of nectar produced (mm) were recorded. Nectar production was measured using microcapillary tubes (Drummond Scientific Co, Broomall, PA 19008) and a ruler, and subsequently converted to volumes (μl). The third, and final, assessment also included a harvest. At harvest, roots and stems were separated using scissors at the soil line. The roots and stems were dried for at least 72 hours in a 60°C drying oven and weighed, in grams, using a scale (Acculab Vicon Digital Scales, Brooklyn, NY 11234).

Statistical Analysis

Temperature, humidity, and CO₂ levels in ambient (control) vs. elevated (treatment) conditions were evaluated using independent sample t-tests.

Plant height, number of immature leaves, and number of fully developed leaves in response to differing CO₂ levels were independently analyzed with nested repeated measures analysis of covariance. In each analysis, the independent variables were: treatment (ambient vs. elevated), chamber nested within treatment, and position in chamber (front vs. back). Trial (1 vs. 2) was entered as a covariate. The repeated measure was: time (measurement 1 vs. 2 vs. 3). All first order interactions, with time,

were also evaluated in the analysis: time*treatment, time*chamber [treatment], time*position in chamber, and time*trial.

Extrafloral nectary numbers and extrafloral nectar volumes were independently analyzed with nested analyses of covariance. In both analyses, the independent variables were: treatment (ambient vs. elevated), chamber nested within treatment, and position in chamber (front vs. back). Plant height, number of immature leaves, number of fully developed leaves, and trial (1 vs. 2) were all entered as covariates.

Finally, stem weights and root weights were independently analyzed with nested analyses of covariance. In both analyses, the independent variables were: treatment (ambient vs. elevated), chamber nested within treatment, and position in chamber (front vs. back). Plant height, number of immature leaves, number of fully developed leaves, and trial (1 vs. 2) were all entered as covariates.

All analyses were conducted using JMP Pro 12.1.0 (SAS Institute Inc. 2015).

Results

There were clear differences between the two chambers (ambient and elevated) in temperature ($t_{14}=9.88$, $P<0.0001$) as well as CO₂ concentration ($t_{14}=25.00$, $P<0.0001$) (Figure 2, Figure 3). There was no significant difference, however, in humidity, between ambient and elevated CO₂ chambers ($t_{14}=1.79$, $P=0.095$).

Plant height and number of fully developed leaves were not significantly different between treatments, however, there were significant differences for both of these traits between trials (Table 1) as plants in the second trial were older and, as a result, more mature. Number of immature leaves were not significantly different between treatments ($F_{1,22}=0.61$, $P=0.44$) or trials ($F_{1,22}=0.14$, $P=0.71$) (Table 1). There were no significant differences in the number of EFNs between treatments for the first or second week (Table 2). On the third week, however, there was a significant difference in the number of EFNs between treatments, as well as a significant difference in the number of fully developed leaves, which was used as a covariate (Figure 4). Plants grown in elevated CO₂ concentrations had higher numbers of EFNs than those grown under ambient CO₂ conditions. There was no significant difference between trials except for the second week of measurements (Table 2).

There was no significant difference in nectar production or its covariates between treatments in the first week of measurements (Table 3). During the second and third weeks of measurement, however, there was a significant difference between ambient and elevated treatments (Figure 5, Figure 6). Plants grown in elevated CO₂ concentrations had higher nectar secretion rates than those grown under ambient CO₂ conditions. During the second week of measurements, there was a significant difference between trials, but by

the third week of measurements no significant difference between trials was present (Table 3).

Stems had significantly less biomass in the elevated CO₂ chambers than the ambient chambers ($F_{1,22}=4.69$, $P=0.041$) and also differed between trials ($F_{1,22}=109.36$, $P<0.0001$) and chambers ($F_{1,22}=4.37$, $P=0.0047$) (Figure 7). When roots were weighed, they differed significantly between trials ($F_{1,22}=13.35$, $P=0.0014$) and between chambers ($F_{1,22}=3.49$, $P=0.014$). There was no significant difference between treatments for root biomass ($F_{1,22}=0.98$, $P=0.33$).

Discussion

The objective of this project was to better understand how increasing CO₂ levels, associated with global climate change, alters plant growth/defense tradeoffs. Using Broad bean plants as a model system, it was discovered that CO₂ concentrations, such as those that are anticipated to occur in the year 2100, directly altered both plant growth and defense. Plants grown under elevated CO₂ had lower biomass (decreased stem weights) but increased defensive capabilities (more EFNs and higher extrafloral nectar secretion rates).

There was no difference in plant traits such as plant height, number of immature leaves, and number of fully developed leaves when plants were grown under ambient or elevated CO₂, suggesting that Broad bean plants grown under elevated CO₂ don't use the extra CO₂ for growth. The stem biomass results further support this conclusion. Previous studies have shown that plants, such as soybeans, in ambient CO₂ produce larger stem biomass than plants under elevated CO₂ conditions (Gray & Brady 2016). If plants grown under elevated CO₂ conditions don't incorporate additional CO₂ into plant tissue, it is possible that additional resources may be incorporated and expressed through defensive traits.

Several studies have examined plant-insect interactions in response to elevated CO₂. Increased plant defenses are believed to be due to hormonal cues during photosynthesis rather than in response to herbivore damage (Zavala et al. 2016). Herbivores, however, are generally attracted to plants with a higher carbon content making the plants more susceptible to herbivory (Zavala et al. 2016, Stiling et al. 2013).

Poor plant quality, as shown here in Broad bean, may result in slower herbivore growth, putting herbivores at increased risk of predation and parasitism (Stiling et al. 2013).

A decrease in plant quality could also have large effects on agricultural yields. Many studies have examined the relationship between elevated CO₂ and agricultural yields, mostly in rice crops. One study revealed that there was no significant difference in rice yields in response to elevated CO₂ alone, but when elevated CO₂ was combined with an increase in temperature, there was a significantly lower yield (Figueiredo et al. 2014). Even more problematic, it is being discovered in crops like soybean that, when exposed to elevated levels of CO₂, previous genetic modifications to the plants are being rendered less effective (Ziska 2010). Many soybean cultivars have been genetically modified to be Roundup ready; as the plants adapt to their changing environment, this modification has become less effective thereby creating a cultivation problem (Ziska 2010). As CO₂ levels continue to rise, similar unexpected problems are likely to arise thereby creating challenges for our global food supply.

When looking at trends in the expression of defensive traits, it appears that plants are putting additional energy into their defensive mechanisms under elevated CO₂ (Lüttge 2013). In the experiment presented here, there was a lack of treatment differences during the first week for both EFNs and nectar. There was also no difference between trials, further suggesting that early in development plants use the majority of their resources for growth. As the plants develop, defense mechanisms such as nectar production are up-regulated. While an increase of EFNs wasn't seen in the second week, plants were clearly stressed, based on the up-regulated nectar production from existing nectaries. By the final

measure, both EFN numbers and nectar levels had significantly increased under elevated CO₂ conditions.

In conclusion, Broad bean plants grown under elevated CO₂ invested more in defense (EFN numbers and extrafloral nectar production) than growth (biomass). With ever-increasing levels of CO₂ in the atmosphere, associated with global climate change, this greenhouse gas could have far-reaching effects on ecosystem dynamics. If policies are not swiftly implemented to ameliorate this global crisis, our food security could be threatened.

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Table 1. Effect of elevated CO₂ concentrations on plant height, number of fully developed leaves, and number of immature leaves

Variable _(df)	Plant Height		Full Leaves		Immature Leaves	
	F	p	F	p	F	p
Treatment _(1,22)	0.001	0.98	0.02	0.88	0.61	0.44
Chamber[Treatment] _(6,22)	1.41	0.26	1.73	0.16	1.05	0.42
Position in Chamber _(1,22)	0.13	0.73	0.45	0.51	0.87	0.36
Trial _(1,22)	13.11	0.0015	134.5	<0.0001	0.14	0.71
Time _(2,21)	21.77	<0.0001	3.87	0.037	2.51	0.11
Time*Treatment _(2,21)	1.09	0.35	1.41	0.27	0.27	0.77
Time*Chamber[Treatment] _(12,42)	0.99	0.47	0.75	0.70	1.72	0.098
Time*Position in Chamber _(2,21)	1.98	0.16	0.68	0.52	1.43	0.26
Time*Trial _(2,21)	46.87	<0.0001	38.79	<0.0001	8.65	0.0018

Bolded values indicate significant [$p < 0.05$] effects

Table 2. Effect of elevated CO₂ concentrations on extrafloral nectary numbers

Variable _(df)	1 st Assessment		2 nd Assessment		3 rd Assessment	
	F	p	F	p	F	p
Treatment _(1,19)	0.50	0.49	3.01	0.099	8.40	0.0092
Chamber[Treatment] _(6,19)	0.51	0.79	1.04	0.43	1.73	0.17
Position in Chamber _(1,19)	0.75	0.40	1.05	0.32	0.023	0.88
Plant Height _(1,19)	0.50	0.49	2.77	0.11	0.74	0.40
Immature Leaves _(1,19)	0.49	0.49	0.026	0.87	0.35	0.56
Full Leaves _(1,19)	0.0022	0.96	0.68	0.42	11.09	0.0035
Trial _(1,19)	3.26	0.087	5.17	0.035	0.92	0.35

Bolded values indicate significant [$p < 0.05$] effects

Table 3. Effect of elevated CO₂ concentrations on nectar production

Variable _(df)	1 st Assessment		2 nd Assessment		3 rd Assessment	
	F	p	F	p	F	p
Treatment _(1,19)	0.51	0.48	12.41	0.0023	6.18	0.022
Chamber[Treatment] _(6,19)	1.00	0.45	1.27	0.32	0.76	0.61
Position in Chamber _(1,19)	0.74	0.40	0.014	0.91	0.21	0.65
Plant Height _(1,19)	0.0071	0.93	0.72	0.41	0.44	0.51
Immature Leaves _(1,19)	0.0062	0.94	2.25	0.15	4.24	0.053
Full Leaves _(1,19)	1.31	0.27	2.38	0.14	0.026	0.87
Trial _(1,19)	2.67	0.12	10.81	0.0039	1.17	0.29

Bolded values indicate significant [$p < 0.05$] effects



Figure 1. The AtmoSim2100; an elevated CO₂ atmospheric simulator

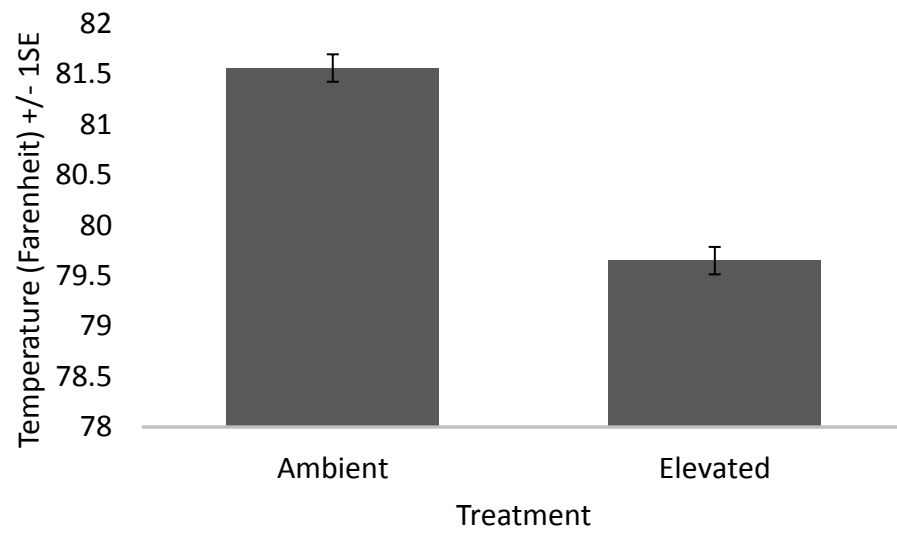


Figure 2. Mean temperatures in ambient and elevated CO₂ treatments (note these values are non-metric, as the Sentinel Analyzer output is non-metric)

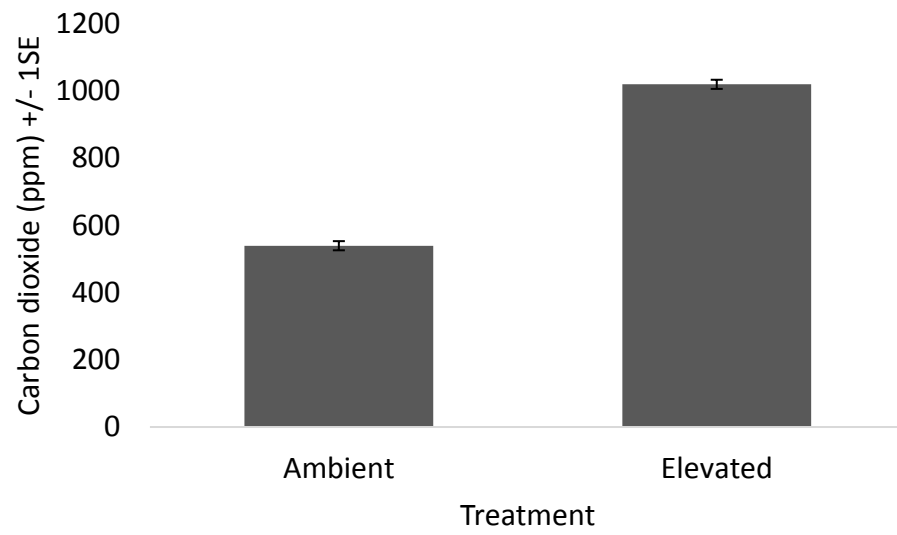


Figure 3. Mean CO₂ concentrations in ambient and elevated CO₂ treatments

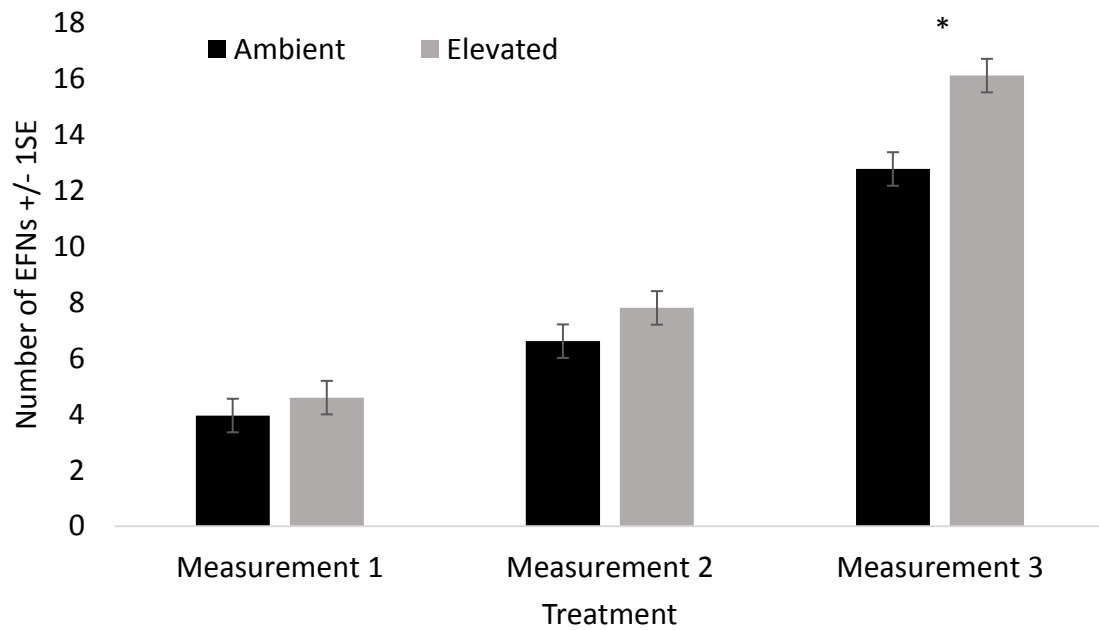


Figure 4. EFN numbers in ambient and elevated conditions, over all three measurements (asterisks over pairs of bars indicate significant differences, $p \leq 0.05$)

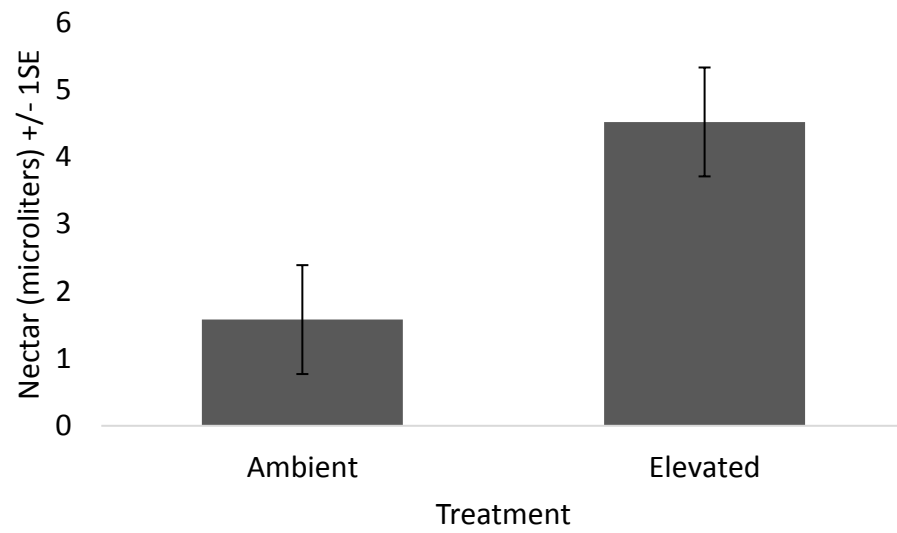


Figure 5. Mean nectar volume (μL) in ambient and elevated CO_2 treatments, on the third measurement

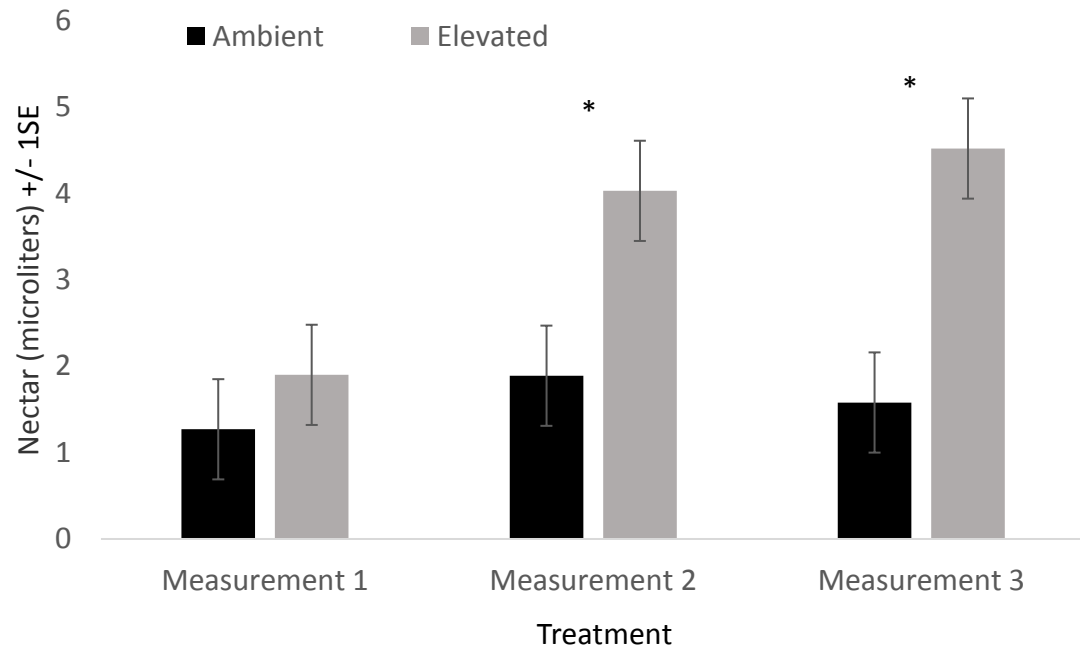


Figure 6. Nectar production in ambient and elevated conditions, over all three measurements (asterisks over pairs of bars indicate significant differences, $p \leq 0.05$)

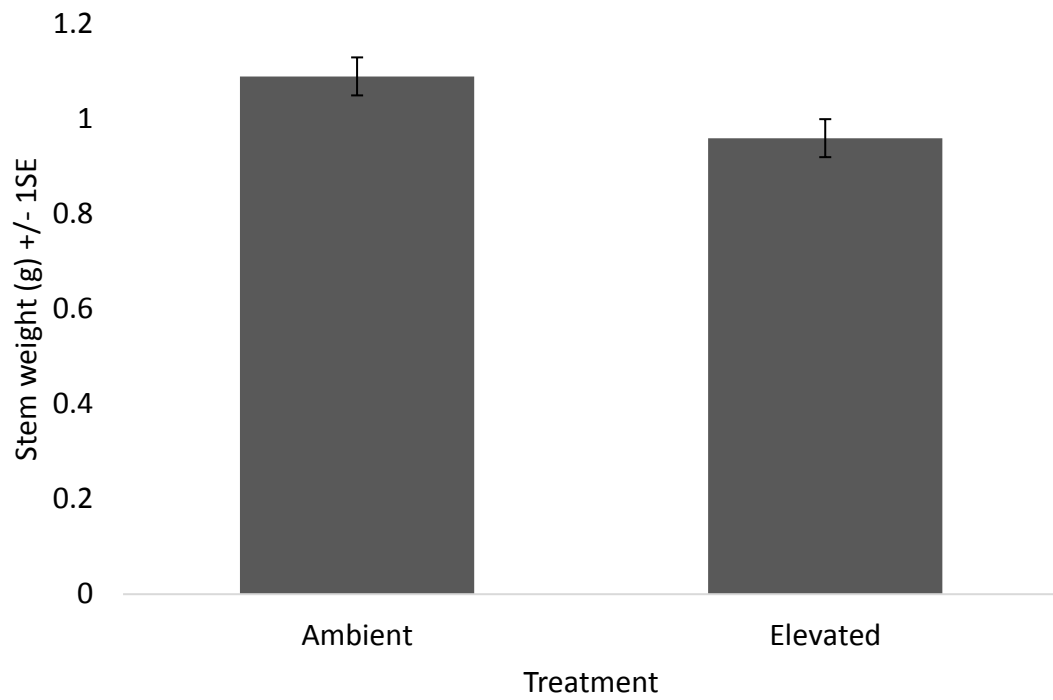


Figure 7. Mean stem dry weights in ambient and elevated CO₂ treatments, after harvest