The effects of elevated carbon dioxide and increased temperature on the growth and forage quality of *Asclepias curassavica* (butterfly weed) grown as a food source for *Danaus plexippus* (monarch butterfly) larvae.

[redacted]

Triad #33

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Abstract

Greenhouse gasses are being released into the atmosphere due to the human use of fossil fuels. The release of these gasses is causing an increased level of carbon dioxide and global warming. This experiment's objectives were to determine the effect of elevated carbon dioxide, the effect of increased temperature, and the effect of carbon dioxide as varying with temperature on the growth of *Asclepias curassavica* and the nutritional quality of *Asclepias curassavica* for *Danaus plexippus*. *Danaus plexippus* was selected because it is a distinctive pollinator and *Asclepias curassavica* was selected because was an available member of *Asclepiadaceae*, which is the host plant of the *Danaus plexippus* larvae. A two-way factorial experiment was conducted with factors of ambient (400 ppm) and elevated (600 ppm) carbon dioxide and average (25 ºC) and increased (30 ºC) temperature. *Asclepias curassavica* plants grown under these conditions had their height measured to observe their growth. The plant's nutritional quality was observed indirectly by feeding the leaves to *Danaus plexippus* larvae and measuring the larvae's growth rate. The effect of carbon dioxide on the growth rate and nutritional quality of *Asclepias curassavica* did not depend on temperature. The temperature did not significantly affect the growth rate and nutritional quality of *Asclepias curassavica* . The level of carbon dioxide did have an affect upon both the on the growth rate and nutritional quality of *Asclepias curassavica*. This means the *Asclepias curassavica* were smaller and less nutritious only due to the carbon dioxide. This agrees with some studies, but disagrees with others. The experiment suggests that rising carbon dioxide will cause smaller and less nutritious plants, which will result in lower growth rates for the organisms that feed upon them.

Introduction

The human use of fossil fuels has lead to the release of greenhouse gasses into the atmosphere. This addition of greenhouse gasses, including carbon dioxide, has affected the earth in several ways (Fritschi *et al.* 1999). The level of carbon dioxide in the atmosphere, along with the rest of the greenhouse gasses, has risen. The added greenhouse gasses, especially carbon dioxide, contribute to global warming (Fritschi *et al.* 1999)

The level of carbon dioxide is expected to increase to 700 ppm within the next century (Coviella and Trumble 1999). The elevated level of carbon dioxide is expected to have several effects upon plants. One effect is an increased growth rate in the plants due to an increase in photosynthesis, which is also accompanied by an increase in carbohydrates (Hughes and Bazzaz 2001). The increased carbohydrates increase the concentration of carbon relative to the nitrogen in the plant when nutrients are limited (Coviella and Trumble 1999). The changed carbon to nitrogen ratio decreased the amount of nitrogen in the plant and leaves (Coviella and Trumble 1999).

Global warming is expected to increase the earth's temperature. Each plant has a temperature which is most favorable to their growth (Moore and Nowak 1998). Once temperatures go above this temperature, the plants are negatively affected (Moore and Nowak 1998). The amount of nutrients in the soil will also affect the rapid growth rate produced by the increased temperature (De Jong *et al.* 1998). The rapid growth continues until all of the available nutrients are consumed (De Jong *et al.* 1998). The growth rate is then reduced (De Jong *et al.* 1998). This depletion of nutrients also reduces the nutritional concentration of the plants (Buse *et al.* 1998). The nutritional concentration reduction can include reduced foliage nitrogen and a toughening of the leaves (Buse *et al.* 1998).

Since both the atmospheric carbon dioxide level and temperature are increasing, both of these factors are considered in this experiment. The effect of this combination will vary from species to species. In some cases, the plants are not affected at all. In *Abutilon theophrasti* and *Amaranthus retroflexus*, the combination of elevated carbon dioxide and increased temperature did not significantly affect the rate of growth in either species (Coleman and Bazzaz 1992).

However, the effect of elevated carbon dioxide and increased temperature should not only be considered in the effect that it has on plants, but also any changes that occur in the plants will affect the organisms that feed upon them should be investigated. *Danaus plexippus* feed only upon the *Asclepiadaceae* family of plants in its larval stage (Mattila and Otis 2003). *Danaus plexippus* as a pollinator is unique and known for its large migration pattern (Eanes and Koehn 1978). The *Danaus plexippus* living east of the Rocky Mountains migrate through Texas to Mexico (Eanes and Koehn 1978). Some travel for thousands of miles to reach their destination (Eanes and Koehn 1978). *Danaus plexippus* pollinate plants along its migration route. The member of *Asclepiadaceae* selected to be the food source for the *Danaus plexippus* in this experiment was *Asclepias syriaca. Asclepias syriaca* was selected as the food source because of its natural abundance and native status. For example, *Asclepias syriaca* was found along 71% of the roadways in Iowa (Hartzler and Buhler 2000). However, *Asclepias curassavica* was used instead. This change was due to an inability to obtain *Asclepias syriaca.*

There were three main objectives for this experiment. The first was to determine the effect of carbon dioxide on the growth of *Asclepias curassavica* and the nutritional quality of *Asclepias curassavica* for *Danaus plexippus*. The hypothesis for this objective was elevated carbon dioxide would affect the growth of *Asclepias curassavica* and the nutritional quality of *Asclepias curassavica* for *Danaus plexippus.* The second was to determine the effect of temperature on the growth of *Asclepias curassavica* and the nutritional quality of *Asclepias curassavica* for *Danaus plexippus*. The hypothesis for this objective was increased temperature would affect the growth of *Asclepias curassavica* and the nutritional quality of *Asclepias curassavica* for *Danaus plexippus.* The final objective was to determine the effect of carbon dioxide on the growth of *Asclepias curassavica* and the nutritional quality of *Asclepias curassavica* for *Danaus plexippus* as varying with temperature. The hypothesis for this objective was the effect of elevated carbon dioxide on the growth of *Asclepias curassavica* and the nutritional quality of *Asclepias curassavica* for *Danaus plexippus* would depend on the temperature.

Methods

To test the objectives of this experiment, *Asclepias curassavica* were grown in a two-way factorial experiment using carbon dioxide and temperature as the factors. The two dependent variables, growth and nutritional quality, were monitored by measuring the growth of *Asclepias curassavica* and by observing the growth rate of the *Danaus plexippus* larvae.

Sixty *Asclepias curassavica* were obtained in their seedling state from Biogenics due to time constraints that did not allow them to be grown from seeds. The *Asclepias curassavica* were potted into 4 inch pots. The plants were divided into four groups with 15 replications in each. The carbon dioxide treatments began four days after replanting. The treatments were produced using carbon dioxide regulators in two different greenhouses. The greenhouses were set to 600 ppm and 400 ppm for the levels of elevated and ambient carbon dioxide.

The increased temperature treatment was started the same day as the carbon dioxide treatments. The average temperature was started a week later, due to supplies not arriving at the same time. Until the actual average temperature treatment was set up, the average temperature plants were kept at the greenhouse room temperature. The actual treatments were produced by placing the *Asclepias curassavica* on propagation mats (Redi-Heat and Progro propagation mats, Hummert International). These mats were set to either 25 ºC for the average temperature or 30 ºC for the increased temperature. Once a week, the *Asclepias curassavica* were measured from the top of pot to the bottom of the highest apical meristem.

Forty *Danaus plexippus* larvae were obtained 3 weeks after the plants were repotted. Each larva was separated into an individual container that was designated as one of the four treatment groups. Each of the four groups of larvae were fed leaves from *Asclepias curassavica* in the corresponding treatment group that were taken off of random plants. Measurements were taken daily of larval weight, the leaf weight given to the larvae, and the weight of leaf material leftover from the previous day. The daily measurements continued for each larva until it pupated. Growth rates of the larvae were determined for each larva by dividing the larva's weight gain by the amount of food that it consumed times the number of days.

Data obtained from measuring the plants and the larvae was analyzed using the JMP software. The JMP software was used to run a 2-way ANOVA on the effect of carbon dioxide and temperature. The analysis used an $\alpha = .05$.

Results

The effect of elevated carbon dioxide on the height of *Asclepias curassavica* did not depend on the temperature ($F = .1060$, $p = .7459$). The plants showed the same decrease in growth when put in elevated carbon dioxide whether they were at average or increased temperature

(See Table 1). There was a significant decrease in height due to elevated carbon dioxide level ($F = 17.1585$, $p = .0001$). There was not a significant change in height due to altering the temperature ($F = .0038$, $p = .9509$).

The effect of carbon dioxide on daily growth rate of *Danaus plexippus* was not dependant upon the temperature ($F = .0005$, $p = .9822$). The daily growth rate showed the same decrease in elevated carbon dioxide whether the larvae where given leaves from average or increased temperature plants (See Table 1). The concentration of carbon dioxide did have a significant effect on the growth rate ($F = 5.7626$, $p = .0217$). The temperature did not have a significant effect upon the larval growth rate ($F = .0082$, $p= .9282$).

An interesting phenomenon that was observed in the *Asclepias curassavica* was a difference in the number of plants that had flowered by the end of the experiment. The effect of carbon dioxide on the number of plants that flowered did not depend upon the temperature ($F= .0000$, $p = 1.000$). There was a decrease in the number of plants that flowered in both increased and average temperature when the level of carbon dioxide was elevated (See Table 2). The number of plants that had flowered by the end of the experiment was significantly influenced by the level of carbon dioxide ($F = 7.1429$, $p= .0098$). Temperature did not significantly effect the end number of plants that had flowered ($F = 1.1429$, $p = .2896$).

A slight difference in the cocooning time of the *Danaus plexippus* was also noted. The day that the first caterpillar cocooned on was termed day one for the data used. The effect of carbon dioxide on the day of cocooning did not significantly depend upon the temperature ($F = 1.4516$, $p = .2361$). The number of days until the larvae cocooned increased for both average and increased temperature when the level of carbon dioxide was elevated (See Table 2). The day of cocooning was significantly affected by the level of carbon dioxide ($F = 8.8323$, $p = .0052$).

The temperature did not have a significant effect on the number of days it took the larvae to cocoon ($F = .1613$, $p = .6903$).

Discussion

The hypothesis of the first objective was supported. The elevated carbon dioxide level created a significant decrease in the growth of *Asclepias curassavica* and the nutritional quality of *Asclepias curassavica* for *Danaus plexippus* (See Figure 1 and Figure 2).

The hypothesis of the second objective was unsupported. The increased temperature did not have a significant effect on the growth of *Asclepias curassavica* and the nutritional quality of *Asclepias curassavica* for *Danaus plexippus* (See Figure 1 and Figure 2).

The hypothesis of the final objective was unsupported by the experiment. The effect of elevated carbon dioxide on the growth of *Asclepias curassavica* and the nutritional quality of *Asclepias curassavica* for *Danaus plexippus* did not depend on the temperature (See Figure 1 and Figure 2).

Biologically, this means that as the *Asclepias curassavica* were smaller and less nutritious only due to elevated carbon dioxide. Temperature did not compound or reduce carbon dioxide's effect nor did it have an effect itself. The decreased nutritional value of *Asclepias curassavica* grown under elevated carbon dioxide resulted in a smaller daily growth rate for the *Danaus plexippus* feeding upon them. So the larvae fed leaves grown in elevated carbon dioxide grew less for the same amount of time.

There were a few unexpected phenomena noted during this experiment. The number of *Asclepias curassavica* that had flowered by the end of the experiment was significantly influenced by the carbon dioxide level. The ambient level of carbon dioxide had twice the number of plants flowering in the elevated level (See Table 2). This seems to indicate that the peak flowering time will occur later as the level of carbon dioxide increases. The day that the *Danaus plexippus* larvae cocooned on was also affected by the carbon dioxide level. The day of cocooning increased as the level of carbon dioxide increased (Figure 3). These two phenomena could affect the success of pollination. If the plants flower later or pollinators emerge later, then the maximum blooming of flowers may not coincide with the maximum amount of pollinators. This might result in some of the plants not being pollinated and some of the pollinators not having enough food. The organisms that depend on plants would be affected as well if the plants were not pollinated.

The hypothesis being unsupported for the interactive effect is in line with some of the studies that have been done. In a study by Coleman and Bazzaz (1992) of *Abutilon theophrasti* and *Amaranthus retroflexus*, the rate of growth in either species was not significantly influenced by the combination of temperature and carbon dioxide.

The nutritional quality hypothesis for carbon dioxide being supported is inline with several other studies that have been conducted with elevated carbon dioxide. The nutritional quality of *Plantago lanceolata* was reduced in 700 ppm carbon dioxide conditions (Fajer 1989; Fajer *et al.* 1991). This was apparent through the decreased biomass of *Junonia coenia*, the buckeye butterfly, fed leaves grown in elevated carbon dioxide (Fajer 1989; Fajer *et al.* 1991).

The finding of no significance for the temperature is dissimilar to the findings of a study with *Operophtera brumata*, the winter moth. In that study, the *Operophtera brumata* had a reduced pupal mass when fed leaves from *Quercus robu* raised at an elevated temperature (Buse *et al.* 1998). The lower pupal masses were the partially caused by a reduction in the amount of leaf nitrogen (Buse *et al.* 1998).

This experiment found that plants will have a lower growth rate and nutritional quality in elevated carbon dioxide, but temperature did not change this effect or have an effect of its own. This suggests that the nitrogen concentration maybe changing, but that temperature is not causing it. The lower nutritional quality does affect the animals that feed upon it, as seen in the daily growth rate. This in turn will affect the plants that rely upon these organisms for pollination. Also, animals that rely on these affected organisms for a source of food are likely to be affected by the decrease in growth rate. From there, the possible affects spiral outward as more plants and organisms are affected. In addition to this, if one species is affected by the carbon dioxide, like the *Danaus plexippus* and another organism is not, any competition going on between the species will be shifted to a new equilibrium.

In the future, it might be interesting to heat the *Asclepias curassavica* in a different fashion. The use of propagation mats heats the plants from below, like soil warming. It might be prudent to heat the plants in a different fashion, say by heat lamps, to simulate the effect of a higher air temperature. Also, the observed timing phenomena should be observed in more detail to see if it is a significant event or not. This experiment was not expecting to see these phenomena and thus was unprepared to properly record them to determine if they were significant.

In concluding, it appears that the temperature will not affect *Asclepias curassavica*'s growth or nutritional quality, either by itself or in changing the effect of carbon dioxide. However, carbon dioxide does show an ability to decrease growth and nutritional quality.

References

Buse, A., J.E. Good, S. Dury, and C.M. Perrins. 1998. Effects of elevated temperature and carbon dioxide on the nutritional quality of leaves of oak (*Wuercus robur L.*) as food for the winter moth (*Operophtera brumata L.*). *Functional Ecology* 12: 742-749.

Coleman, J.S. and F.A. Bazzaz. 1992. Effects of CO_2 and temperature on growth and resource use of co-occurring C_3 and C_4 annuals. *Ecology* 73: 1244-1259.

Coviella, C.E. and J.T. Trumble. 1999. Effects of elevated atmospheric carbon dioxide on insect-plant interactions. *Conservation Biology* 13: 700-712.

De Jong, T.J., L.G. Roo, and P.G. Klinkhamer. 1998. Is the threshold size for flowering in *Cynoglossum officinale* fixed or dependent on environment? New Phytologyist 138: 489-496.

Eanes, W.F. and R.K. Koehn. 1978. An analysis of genetic structure in the monarch butterfly, *Danaus plexippus L. Evolution* 32: 784-797.

Fajer, E.D. 1989. The effects of enriched CO_2 atmosphere on herbivore interactions: growth responses of larvae of the specialist butterfly, *Junonia coenia* (Lepidoptera: *Nympalidae*). *Oecologia* 81: 514-520.

Fajer, E.D, M.D. Bowers, and F.A. Bazzaz. 1991. The effects of enriched CO_2 atmospheres on the buckeye butterfly, *Junonia coenia*. *Ecology* 72: 751-754.

Fritschi, F.B., K.J. Botote, L.E. Sollenberger, and L.H. Allen Jr. 1999. Carbon dioxide and temperature effects on forage establishment: tissue composition and nutritive value. *Global Change Biology* 5: 743-753.

Hartzler, R.G. and D. D. Buhler. 2000. Occurrence of common milkweed (Asclepias syriaca) in croplands and adjacent areas. *Crop Protection* 19: 363-366.

Hughes, L. and F.A. Bazzaz. 2001. Effects of elevated CO_2 on five plant-aphid interactions. *Entomologia Experimentalis et Applicata* 99: 87-96.

Mattila, H.R. and G.W. Otis. 2003. A comparison of the host preference of monarch butterflies (*Danaus plexippus*) for milkweed (*Asclepias syriaca*) over dog strangler vine (*Vincetoxicum rossicum*). *Entomologia Experimentalis et Applicata* 107: 193-199.

Moore, D.J. and R.S. Nowak. 1998. Photosynthetic acclimation to temperature and drought in the endemic Chelan rockmat, *Petrophyum cinerascens* (*Rosaceae*). *The American Midland Nauralist* 139: 374-382.

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Table 1 The means ± standard errors of *Asclepias curassavica* height increase and of *Danaus plexippus's* daily growth rate in the four treatment groups of the two way factorial experiment with factors of carbon dioxide, 400 ppm and 600 ppm, and temperature, 25 ºC and 30 ºC.

	Ambient Carbon Dioxide & Average Temperature	Ambient Carbon Dioxide & Increased Temperature	Elevated Carbon Dioxide & Average Temperature	Elevated Carbon Dioxide & Increase Temperature
Daily Growth Rate of Larvae (g/(g*day))	0.009620 ± 0.0007794	0.009712 ± 0.0007621	0.007689 ± 0.0009946	0.007744 ± 0.0006786
Plant Height Increase (cm)	8.74 ± 1.178	8.43 ± 0.771	5.13 ± 0.503	5.34 ± 0.615

Table 2 The phenomena observed during the experiment. The number of *Asclepias curassavica* that had flowered by the end of experiment and the means ± standard errors of the day of cocooning for the *Danaus plexippus* larvae in the four treatment groups of the two way factorial experiment with factors of carbon dioxide, 400 ppm and 600 ppm, and temperature, 25 ºC and 30 ºC. The day that the first larva cocooned on was called day one.

	Ambient Carbon Dioxide & Average Temperature	Ambient Carbon Dioxide & Increased Temperature	Elevated Carbon Dioxide & Average Temperature	Elevated Carbon Dioxide & Increase Temperature
Number of Plants Flowering at End of Experiment	9	11	4	6
Mean Day of Cocooning	3.1 ± .604	4.1 ± .526	5.7 ± .789	5.3 ± .539

Figure 1 The means with standard error bars of *Asclepias curassavica*'s height increase over the entire experiment in the four treatment groups of the two way factorial experiment with factors of carbon dioxide, 400 ppm and 600 ppm, and temperature, 25 ºC and 30 ºC.

Figure 2 The means with standard error bars of *Danaus plexippus*'s daily growth rate in the four treatment groups of the two way factorial experiment with factors of carbon dioxide, 400 ppm and 600 ppm, and temperature, 25 ºC and 30 ºC.

Figure 3 The means with standard error bars of *Danaus plexippus*'s day of cocooning in the four treatment groups of the two way factorial experiment with factors of carbon dioxide, 400 ppm and 600 ppm, and temperature, 25 ºC and 30 ºC. The day the first larvae cocooned was called day one.

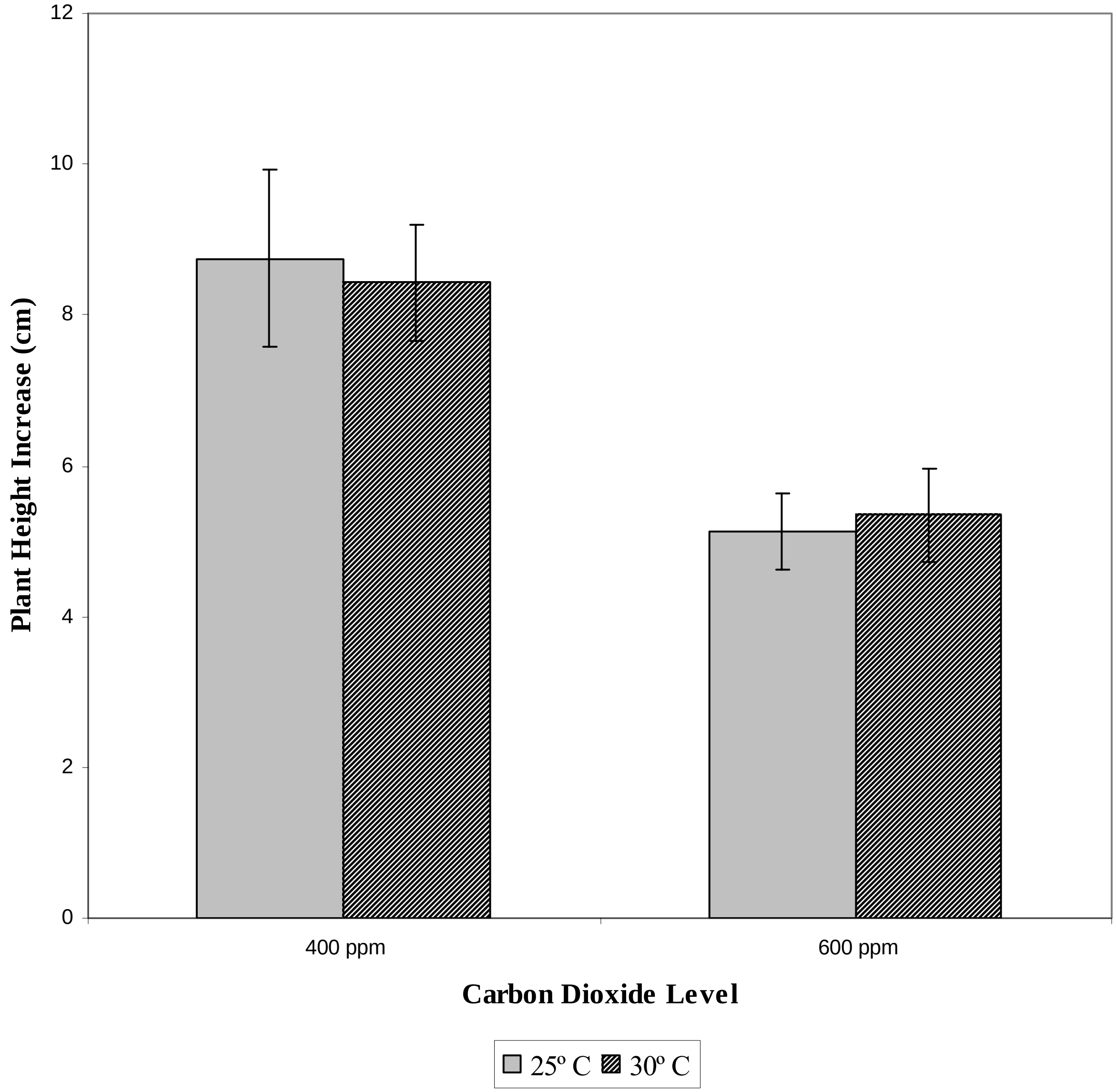

12
10
8
6
4
2
0
Plant Height Increase (cm)
400 ppm
600 ppm
Carbon Dioxide Level
25º C
30º C

Figure 1

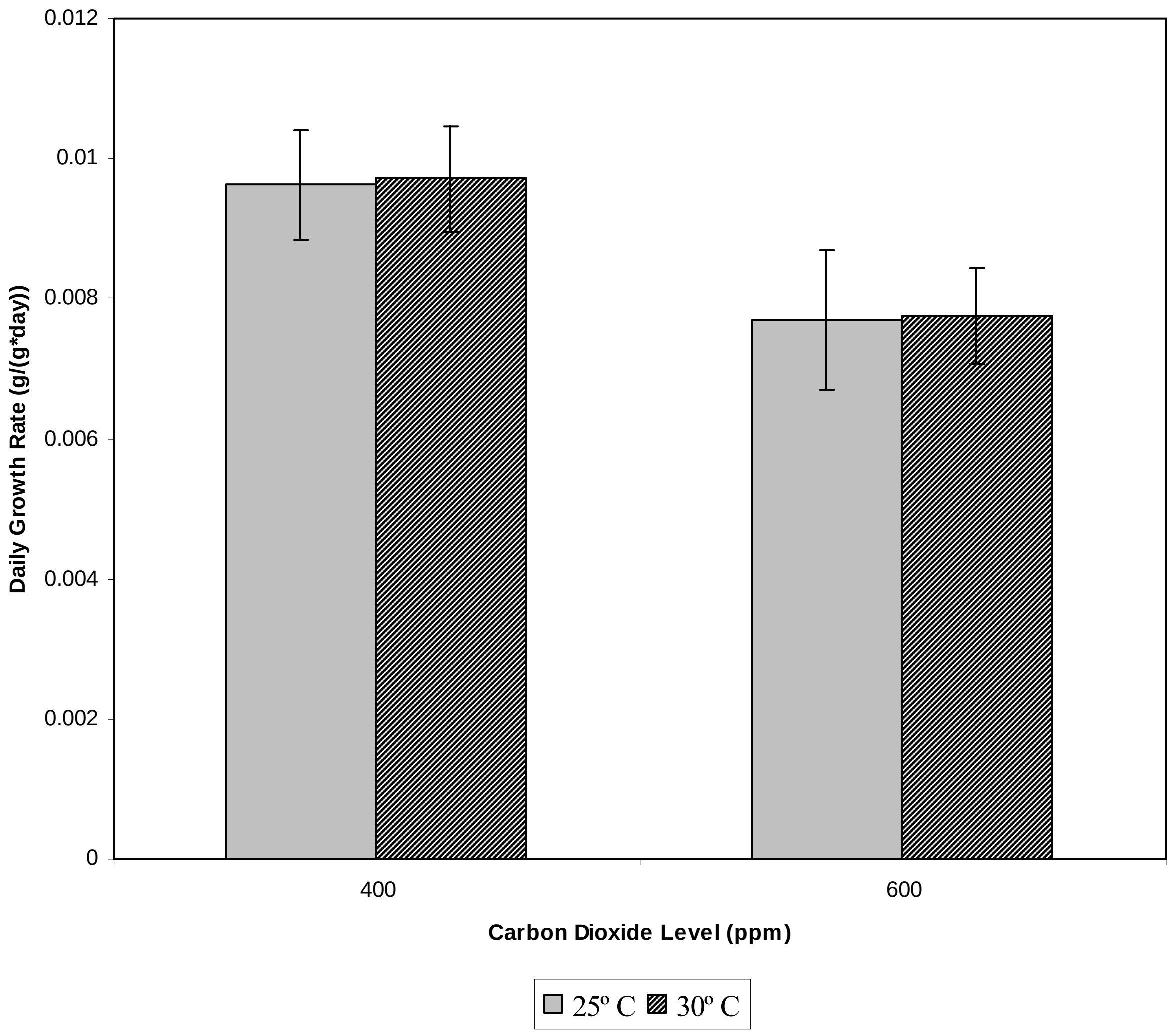
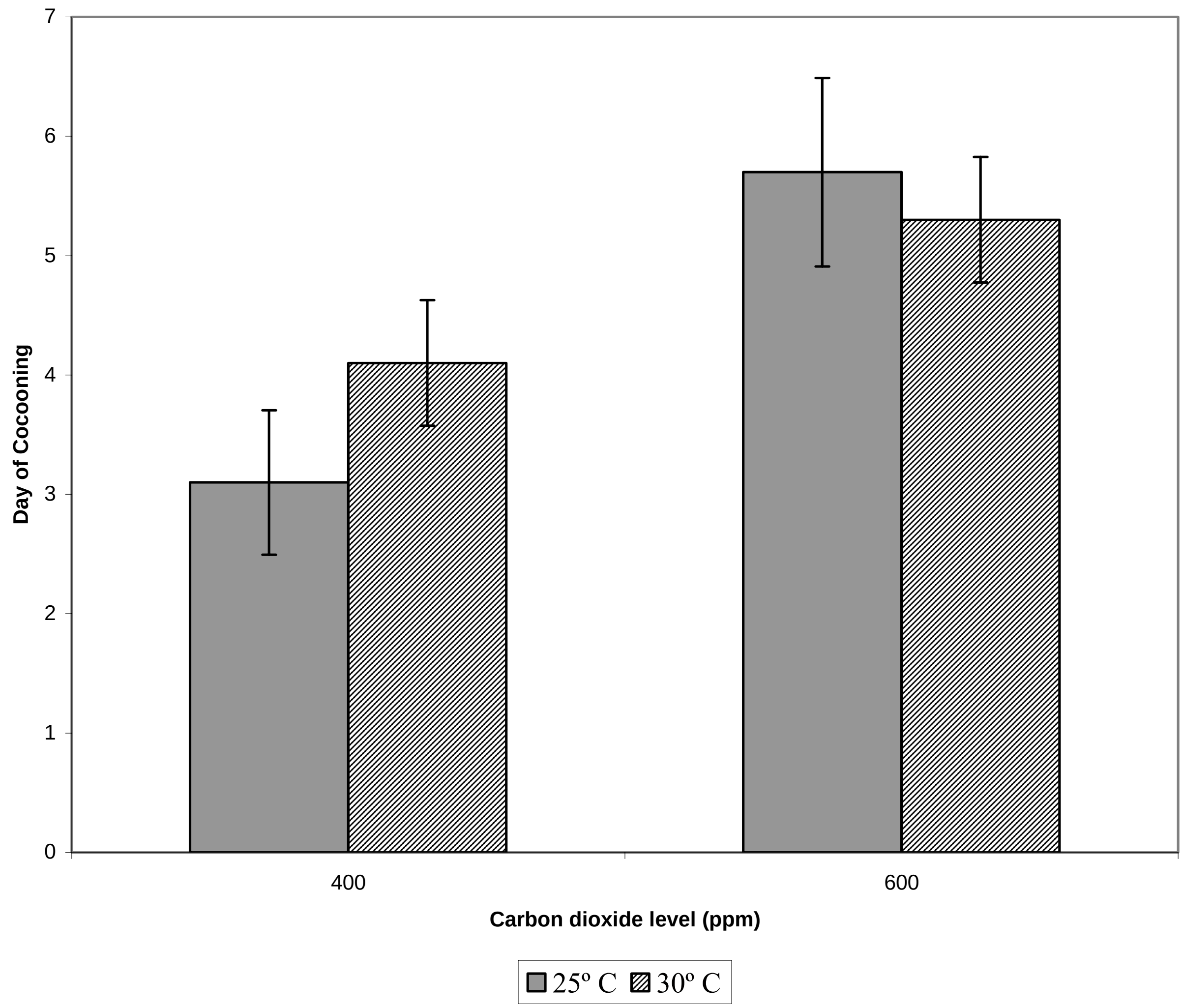

0.012
0.01
0.008
0.006
0.004
0.002
0
Daily Growth Rate (g/(g*day))
400
600
Carbon Dioxide Level (ppm)
25º C
30º C

Figure 2



7
6
5
4
3
2
1
0
Day of Cocooning
400
600
Carbon dioxide level (ppm)
25º C
30º C

Figure 3