THE EFFECTS OF CARBON DIOXIDE, TEMPERATURE, AND WATER CHANGES ON GROWTH AND HEALTH OF GULF RYEGRASS (Lolium)

Sophia Chen, Madeleine Granovetter, Michaela Hitchner, Kyle Huang, Akshay Kadhiresan, Johnna Margalotti, Stephanie Ren, Adeena Samoni, Kelsey Walter

Advisor: Dr. Arun Srivastava
Assistant: Runi Patel

ABSTRACT

The effects of global climate change are at the forefront of the scientific community. One of the Earth’s most important constituents is its vegetation, and the changes that the Earth is undergoing are speculated to affect plant biomass and productivity on a global scale. In this experiment, Gulf Ryegrass (Lolium) was subject to simulated conditions of global climate change to investigate the effects it will have on the plants. These conditions included two scenarios of CO₂ levels (550 ppm and 850 ppm), a lower and higher temperature (29.6°C and 32.0°C), and a lower and higher precipitation scenario (120 mL and 180 mL). The results show that consistent with the hypothesis, the grass blades exposed to higher concentrations of CO₂ grew taller than those of lower concentrations. Similarly, it was found through the experiment that increased temperature does have an adverse effect on plant growth. However, while limiting water does harm growth, providing more water than the average global mean increases the efficiency of productivity.

INTRODUCTION

Climate change over the past 50 years has produced great changes in the distribution and abundance of species and has been principally implicated with a change in temperature; studies suggest a stable temperature is necessary for the maintenance of global biodiversity (1). One mechanism by which temperatures are regulated is the greenhouse effect, in which essential gases such as CO₂, methane, water vapor, nitrous oxide, and ozone trap solar energy in the atmosphere in the form of heat. While this phenomenon is intended to keep the planet at a temperature suitable for life, a significant rise in global temperature due to thicker layers of greenhouse gases, known commonly as global warming, can be detrimental to species and biomes with sensitive temperature requirements (1, 2, 3). Global temperature rise also has adverse effects on Earth’s oceans, as they have been absorbing increasing amounts of heat, causing sea levels to rise due to the expansion of seawater and melting ice caps (2).

CO₂ is one of the most significant greenhouse gases involved in global warming, as CO₂ remains in the atmosphere for longer periods of time than nitrous oxide and methane (4). Prior to the 18th century, atmospheric CO₂ levels fluctuated between 180 and 280 ppm (3); after the Industrial Revolution, the concentration of CO₂ has grown to a current high of 401.14 ppm (2, 3, 4, 5). There are also several natural processes by which CO₂ cycles through ecosystems. Photosynthetic organisms and bodies of water such as oceans absorb CO₂ from the atmosphere (6, 7). Absorbed CO₂ can lower the pH of water and harm aquatic ecosystems that depend on a relatively stable concentration of hydrogen ions. By the end of the next century, the increase in
atmospheric CO$_2$ levels is expected to cause a 50% drop in the surface pH of oceans, resulting in the reduced calcification of marine plankton and diminished overall productivity (8).

Currently, at 401.14 ppm (4), atmospheric CO$_2$ levels are over 100 ppm higher than the levels of the 420,000 years prior to industrialization (3). It is estimated that in the next century, CO$_2$ levels will reach as high as 600 ppm (4). Among many other consequences, the resulting rising temperatures will cause shifting biomes, mass extinctions, and rising ocean levels.

Global climate change not only causes temperature shifts, which negatively impact the biosphere, but it is also predicted to drastically alter weather patterns. Severe weather events such as hurricanes will become more common as the warming trend continues, producing heavy flooding; monsoon conditions are presently on the rise, especially in the tropical regions of Southeast Asia (4,9). In contrast, the emergence of severe weather patterns as a consequence of global warming also has the potential to plague certain regions with unprecedented dry spells (9).

The purpose of this study is to simulate future atmospheric conditions, in order to observe how the health of ryegrass (*Lolium*) and various species of algae would be affected if the perpetuation of global warming continues to accelerate over the next several decades. Crucial to the investigation is the isolation of three variables within contained environments: quantity of CO$_2$, quantity of water, and change in temperature. To create realistic conditions, the manipulation of each variable corresponds with predictions for the climate of the next several decades. The results garnered from these experiments will serve as a measure of the health of domesticated plant species (ryegrass) as well as undomesticated organisms (algae), ultimately providing insight into the reliability of agriculture and the stability of ecosystems in future years.

The first component of the experiment tests the effects of increasing atmospheric CO$_2$ on ryegrass and algae. After being exposed to 400, 550, and 850 ppm of CO$_2$, the respective grass samples are tested for changes in growth patterns, the breakdown of biomass, relative amount of photosynthetic pigments, and fluctuations in soil pH. Algae are examined for levels of dissolved oxygen in addition to changes in pH. It is hypothesized that increased levels of CO$_2$ will subsequently lead to increased plant growth in both the grass and algae. When there is a high concentration of CO$_2$, the grass will be able to undergo a larger degree of photosynthetic carbon fixation, which will likely allow the plant to produce more biomass. As the grass samples will be exposed to two different quantities of CO$_2$, it is presumed that by this logic, the higher amount of CO$_2$ will produce more significant growth. Also, increased CO$_2$ will allow algae to flourish, which will inevitably decrease the amount of dissolved oxygen present in its habitat. The soil pH is also monitored for changes, as CO$_2$ entering the soil as H$_2$CO$_3$ will likely lower the pH.

A second experiment examines the impact of increased temperature on algae and grass growth. Higher temperatures can cause increased transpiration, faulty functioning, and protein denaturation. Experimental grass samples are exposed to two different temperatures as variable. It is hypothesized that higher temperatures will inhibit plant growth for the reasons stated above, and that these results will be exacerbated in the sample exposed to the highest temperatures.

A third experiment tests how different levels of precipitation affect the growth of ryegrass. To simulate both flood and drought conditions, one sample of grass receives...
approximately 21% more water than the average global mean, corresponding with future projections, while another is partially deprived of water, receiving 21% less. Either extreme can be detrimental to the ryegrass, yielding results that demonstrate less vitality than the control. It is hypothesized that in conditions of drought and flood, plant growth will be inhibited.

MATERIALS AND METHODS

Grass Setup

Miracle-Gro Organic Choice Potting Mix (500.0 mL, packed) was added to ten aluminum foil trays (7.88” L x 7.88” W x 1.88” H). Gulf Rye Grass seeds (3.00 g) were sprinkled on the soil and covered evenly with additional soil (100 mL, packed). Tap water (300 mL, pH 7.5) was added to each tray to stimulate growth. The trays were placed in a temperature-controlled incubator (held at 31.6 ± 0.2° C) with consistent lighting. The trays were watered for the next five days with 100 mL, 300 mL, 300 mL, 200 mL, and 150 mL of water (pH 7.0), respectively.

The trays were numbered one through ten and introduced to their respective variables after germination, as shown in Table 1 below. The trays, except for the two involved in the water-variant experiment, continued to receive 150 mL/day until the conclusion of the experiment. In order to balance out the removal of soil each day due to the biomass data collection, the trays were replenished on the third day of data collection with 200 mL of soil, and with 100 mL of soil on subsequent days.

Table 1: Allocation of Trays Based On Variable For Bookkeeping Purposes

<table>
<thead>
<tr>
<th>Variable</th>
<th>Tray 1</th>
<th>Tray 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Tray 1</td>
<td>Tray 2</td>
</tr>
<tr>
<td>CO₂</td>
<td>Tray 4 (More CO₂; 550 ppm)</td>
<td>Tray 5 (Even More CO₂; 850 ppm)</td>
</tr>
<tr>
<td>Temperature</td>
<td>Tray 6 (Lower Temperature; 29.6°C)</td>
<td>Tray 7 (Higher Temperature; 32.0°C)</td>
</tr>
<tr>
<td>Water</td>
<td>Tray 9 (Less Water; 120 mL)</td>
<td>Tray 10 (More Water; 180 mL)</td>
</tr>
</tbody>
</table>

Statistical Analysis

For this series of experiments, we wish to compare true averages for grass growth and come to a statistically significant conclusion, but the population standard deviation (the standard deviation in heights of all existing grass blades) is unknown. Thus, a two-sample t hypothesis test is appropriate. The α, or significance level, is a pre-established threshold probability; the value p is the probability of the trends shown in results happening by chance; thus, if p is calculated as less than α by calculator programs, the results are significant.

CO₂ Setup

Two trays (Trays 4 and 5) were exposed to varying amounts of CO₂ with a chilled CO₂ cylinder. The trays were placed in sealed bins, with a tube providing CO₂. A Vernier CO₂ gas

---

1 Miracle-Gro Organic Choice Potting Mix Contents (Derived from pasteurized poultry litter):
- Nitrogen 0.10% (0.002% Ammoniacal, 0.001% Water Soluble, 0.097% Water Insoluble)
- Available phosphate (P₂O₅)……………………0.05%
- Soluble potash (K₂O)…………………………0.05%
2 Trays 2 and 8 were not subject to experimentation.
3 Vernier dissolved oxygen probe connects to a Vernier Lab Quest.
sensor (Order Code: CO2-BTA) was initially used to calibrate (accurate to within 40 ppm) the appropriate amounts of CO$_2$ released from the tank. The tray with a small increase in atmospheric CO$_2$ underwent this procedure for five minutes each day (550 ppm), simulating atmospheric CO$_2$ in the next 20-35 years. The tray with a larger increase in CO$_2$ levels underwent treatment for ten minutes daily (850 ppm), simulating the atmosphere in 2100. After each sample had been exposed to the proper amount of CO$_2$, the tube was removed and each bin was sealed with tape to prevent gas exchange between the internal and external environments. The control trays (Trays 1 and 3) were exposed to 400 ppm, similar to current CO$_2$ levels. Approximately once every 24 hours, the boxes were opened to take samples; CO$_2$ levels were replenished using the same previous method.

**Temperature Setup**

The control trays (Tray 1 and Tray 3) of grass were kept in the incubator at 31.6 ± 0.2°C. One tray of grass was subject to a lower temperature (Tray 6) while another was subject to a higher temperature (Tray 7). Tray 6 of grass was placed in a closed container and then set into a water bath to hold the container at a constant ambient temperature of about 29.6°C. The temperature was held constant by a Penn-PLRX Cascade Heat sensor (Model: CH850, 110 V ~ 60 Hz, 50 W). Tray 7 was placed in a container with an increased temperature by setting the tray of grass on top of a heat mat terrarium heater (NO.: 13031, 120VAC, 60Hz, 8W). The ambient temperature of the grass was measured using a Stainless Steel Temperature Probe made by Vernier (order code: TMP-BTA) and held constant. This temperature was 32.0°C, which is the projected temperature one decade from now (10).

**Water Setup**

The different grass trays were each watered to simulate the global average of rainfall per surface area. The median average rainfall per year was multiplied by a conversion factor in order to find the proper level of watering for the trays that were used (11, 12):

$$\left(\frac{40.767\text{"rain}}{1\text{year}}\right) \times \left(\frac{17,370,000\text{gallons}}{1\text{mi}^2}\right) \times \left(\frac{3785.41\text{mL}}{1\text{gallon}}\right) \times \left(\frac{1\text{mi}^2}{1\text{gallon}}\right) \times \left(\frac{1\text{year}}{2.59 \times 10^{10}\text{cm}^2}\right) \times \left(\frac{1\text{yr}}{365\text{days}}\right) \times \left(\frac{522.580\text{cm}^2}{1\text{year}}\right) = 148.18\text{mL/day}$$

**Equation 1:** Calculation of the average global mean precipitation as applied to each tray of ryegrass

The controls (Trays 1 and 3) were watered with 150.0 mL/day. A projected 7% increase in precipitation will result from an increase of 1°C, so a 3°C change is predicted over the next 20 years, Trays 9 and 10 were watered with an approximate deviation of ±21%, corresponding to 180.0 mL per day and 120.0 mL per day, respectively (13). The grass in Trays 9 and 10 were allowed to germinate at present-day conditions with the exception of changes in precipitation.

**Density**

The density of the grass, used as a representation of plant yield and health, was calculated in units of blades per cm$^2$ by counting the quantity of grass blades in a section of area 32.4 cm$^2$.

---

4 The ambient temperature was not as high as intended.
5 Vernier dissolved oxygen probe connects to a Vernier Lab Quest.
Height/Growth Rate
The height (cm) was measured by averaging the heights of six random blades per tray. The set of these averages was used to calculate the growth rate, another indication of productivity. The growth rates were then compared to observe the effect of each condition on the grass.

Biomass
A section of soil and grass (32.4 cm² in area) was uprooted, keeping all the vegetation and soil intact. The soil was then removed completely from the roots. The grass and roots were then paper dried, and the wet weight was recorded. The blades of grass were then separated from their roots, and both the blades and roots were inserted in separate bags and placed in an oven overnight for drying at 80°C. The dry weight measurements of the grass blades and roots were taken the next day. These measurements are indicators of the productivity of the grass.

Spectrophotometry of Chloroplasts
A Vernier® SpectroVis Plus Spectrophotometer (Model: SVIS-PL, 380 nm-950 nm) was used to measure the amount of pigment in each sample of grass, which is directly correlated to the number of chloroplasts. A random sample of six blades without roots was taken from each tray and crushed in a mortar and pestle with the addition of 10 mL of water. The sample was examined with the spectrophotometer and the full spectrum of absorption was generated on Days 3 and 6.

Soil pH
A pH testing kit was utilized in order to measure the change in soil pH. Measurements entailed taking a dried soil sample, adding BaSO₄, and injecting a stock solution from a Soil pH, N, P, K Test Kit (Environmental Concepts #1665). The solution was compared to a color chart, and the results were recorded on Days 3 and 6.

Qualitative Observations
The color and plasmolysis of the samples were observed qualitatively each day as indications of health. This was done by comparing each tray to the control trays and noting differences in pigmentation and rigidity of the blades to gauge the relative visual appearances.

Algae Setup
Algae samples were collected from the experimental ecology ponds located outside the Hall of Sciences at Drew University. Algae food (100 mL) was added to the original stock (200 mL) in a 400 mL beaker. This was repeated to make five identical beakers of algae. One beaker was kept with the control trays and subject to those conditions; one with the higher temperature tray; one with the lower temperature tray; one with the higher CO₂ tray; and one with the even higher CO₂ tray. A dissolved oxygen probe made by Vernier® (order code: DO-BTA) was used to measure the dissolved oxygen in the algae beakers (14) on day 3 and day 6. This is proportional to the quantity of algae and an indicator of how much photosynthesis is occurring (15, 16).

---

6 Vernier dissolved oxygen probe connects to a Vernier Lab Quest.
7 Vernier dissolved oxygen probe connects to a Vernier Lab Quest.
RESULTS

CO$_2$-Variant

For the CO$_2$ trays, the control was set to 400 ppm of CO$_2$ with the variable trays containing 550 ppm and 850 ppm. All three trays of grass exhibited a decreasing growth density over the trial period. However, compared to the other trays with 550 ppm and 850 ppm of CO$_2$, the growth density of the control tray (400 ppm) stayed relatively constant. Meanwhile, the grass blade growth densities of both the 550 ppm tray and the 850 ppm tray significantly decreased.

![Effects of CO$_2$ Level on Growth Density](image1)

Figure 1: Growth density of grass samples when exposed to variable CO$_2$, with nonlinear trendlines

Whereas the grass in the control tray (400 ppm) and the 550 ppm tray displayed steady growth and then equally steady decline, the grass in the 850 ppm tray demonstrated overall steady growth. The grass exposed to 850 ppm of CO$_2$ grew approximately 2 cm throughout the 6 experimental days. Interestingly, the grass exposed to 550 ppm of CO$_2$ actually shrunk about 3 cm throughout the 6 days, while the grass in the control tray (400 ppm) stayed relatively constant, growing approximately a centimeter throughout the experimental period.

![Effects of CO$_2$ Level on Average Grass Height](image2)

Figure 2: Average grass blade height when exposed to varying amounts of CO$_2$, with nonlinear trendlines

Statistical Analysis: Control (400 ppm) versus increased CO$_2$ (550 ppm)

$H_0$: $\mu_1 = \mu_2$ , $H_a$: $\mu_1 > \mu_2$

Given that $\mu_1$ is the true mean growth height for the grass treated with 550 ppm and $\mu_2$ is the true mean growth height for the grass treated with 400 ppm, assume that the sample is random, independent, and normal, then run a two-sample T test.
\[ t = -0.6634 \mid p = 0.7378 \mid \bar{x}_1 = 11.505 \mid \bar{x}_2 = 11.920 \mid S_{x1} = 1.2843 \mid S_{x2} = 0.8357 \mid \alpha = 0.05 \]

The p-value, 0.7378, is greater than the significance level of 0.05. There is insufficient evidence to reject the null hypothesis, and maintain that the true average height of grass treated with 550 ppm of CO\(_2\) is not significantly greater than that of grass treated with 400 ppm.

Statistical Analysis: Control (400 ppm) versus highly increased CO\(_2\) (850 ppm)

\[ H_0: \mu_1 = \mu_2, \quad H_a: \mu_1 > \mu_2 \]

Given that \( \mu_1 \) is the true mean growth height for the grass treated with 850 ppm and \( \mu_2 \) is the true mean growth height for the grass treated with 400 ppm, assume that the sample is random, independent, and normal, then run a two-sample T test.

\[ t = 2.2043 \mid p = 0.0264 \mid \bar{x}_1 = 12.898 \mid \bar{x}_2 = 11.920 \mid S_{x1} = 0.6953 \mid S_{x2} = 0.8357 \mid \alpha = 0.05 \]

The p-value, 0.0264, is less than the significance level of 0.05. There is sufficient evidence to reject the null hypothesis, and accept the alternative: the true average height of grass treated with 850 ppm of CO\(_2\) is significantly greater than that of grass treated with 400 ppm.

For the first three days of the experiment, the wet weight of the grass that was exposed to 550 ppm (Tray 4) of CO\(_2\) was very similar to that of the grass that was exposed to 400 ppm (Control) of CO\(_2\). The dry weight of the grass in Tray 4 is very small, with the exception of the data from Day 3, which is unusually large. The biomass of the wet weight of the sample taken from Tray 5, which was treated with 850 ppm of CO\(_2\), is similarly high. In addition, like the other samples, the biomass of the dry blades and roots from this sample is diminutive.

![Figure 3: Biomass of grass and root samples when exposed to A) 400 ppm CO\(_2\); B) 550 ppm CO\(_2\); C) 850 ppm CO\(_2\).](image)

In the spectrophotometric readings, the tray treated with 850 ppm CO\(_2\) possessed higher absorbance readings in the green spectrum (500 to 570 nm), and thus had the healthiest and most numerous chloroplasts. The trays treated with 550 ppm and 400 ppm, meanwhile, possessed much lower absorbance readings (i.e., fewer chloroplasts).
Figure 4: Absorbance spectrums for chloroplasts in CO\textsubscript{2} variant trays with (A) initial values and (B) final values; Tray 1 = Red; Tray 4 = Yellow; Tray 5 = Blue

In the control trays, there was minimal plasmolysis and wilting taking place. However, both trays of increased CO\textsubscript{2} began to deteriorate on Day 4, both in color and plasmolysis.

Table 2: Color and plasmolysis for grass under conditions of a variable amount of CO\textsubscript{2}

<table>
<thead>
<tr>
<th>Control Trays 1 and 3</th>
<th>Carbon Dioxide Trays</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>More CO\textsubscript{2} (550 ppm)</td>
</tr>
<tr>
<td>Day #</td>
<td>Color</td>
</tr>
<tr>
<td>-------</td>
<td>----------</td>
</tr>
<tr>
<td>1</td>
<td>Bright Green</td>
</tr>
<tr>
<td>2</td>
<td>Bright Green</td>
</tr>
<tr>
<td>3</td>
<td>Bright Green</td>
</tr>
<tr>
<td>4</td>
<td>Bright Green</td>
</tr>
<tr>
<td>5</td>
<td>Lighter Green</td>
</tr>
<tr>
<td>6</td>
<td>Lighter Green</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 5: Dissolved oxygen in algae under conditions of an increased amount of CO$_2$

From the CO$_2$ variant trials with algae in Figure 5, the dissolved oxygen in the control with 400 ppm CO$_2$ decreased over time while increased amounts of CO$_2$ led to increased levels of dissolved oxygen. This indicates that an increase in CO$_2$ leads to an increase in algae growth since photosynthesis is the main contributor to dissolved oxygen levels in this experiment.

Temperature-Variant:

The blade density for both the control and increased temperature were relatively constant for the 6 days. However, the growth density decreased in the two higher temperature trays, starting at Day 3. As shown in Figure 6, the density of grass in the control tray overall was less than the density in the other trays, which averaged to be about equal, although the trend lines on Figure 6 indicate a high density growth rate for the 29.6°C tray.

Figure 6: Growth density of the three ryegrass trays due to temperature variances, with nonlinear trendlines

As shown in Figure 7, there is a general trendline for the height of all three grass trays that shows the peak height at Day 4, followed by a decline in growth. Also, there is an increase in growth of the tray at 29.6°C while the 32°C and 31.6°C trays stayed relatively constant for the 6 days. The overall trend lines on Figure 7 indicate an increase in the height of the two lowest temperature ryegrass trays while the higher temperature one declined in growth. The heights for the lower temperature and the constant temperature fluctuated more than for the higher
temperature. The tray that had the highest average height was the 32°C tray, followed by the 29.6°C tray, and finally the 31.6°C tray. An outlier was observed for the Day 2 measurement of the 29.6°C tray where there was a severe drop.

Figure 7: Average grass heights of tray 6 (29.6°C), tray 1 (31.6°C), and tray 3 (32°C) over 6 days, with nonlinear trendlines

Statistical Analysis: Control (31.6° C) versus Low Temperature (29.6° C)

$H_0$: $\mu_1 = \mu_2$, $H_a$: $\mu_1 > \mu_2$

Given that $\mu_1$ is the true mean growth height for the grass kept at 29.6° C and $\mu_2$ is the true mean growth height for the grass kept at 31.6° C, assume that the sample is random, independent, and normal, then run a two-sample T test.

$t = 8.1559 \mid p = 0.2169 \mid \bar{x}_1 = 12.333 \mid \bar{x}_2 = 11.920 \mid S_{x1} = 0.9180 \mid S_{x2} = 0.8357 \mid \alpha = 0.05$

The p-value, 0.2169, is greater than the significance level of 0.05. We thus have insufficient evidence to reject the null hypothesis, and maintain that the true average height of grass kept at 29.6° C is not significantly greater than that of grass kept at 31.6° C.

Statistical Analysis: Control (31.6° C) versus High Temperature (32.0° C)

$H_0$: $\mu_1 = \mu_2$, $H_a$: $\mu_1 < \mu_2$

Given that $\mu_1$ is the true mean growth height for the grass kept at 32.0° C and $\mu_2$ is the true mean growth height for the grass kept at 31.6° C.

Assuming that the sample is random, independent, and normal, run a two-sample T test.

$t = 1.8401 \mid p = 0.9406 \mid \bar{x}_1 = 12.567 \mid \bar{x}_2 = 11.920 \mid S_{x1} = 0.2066 \mid S_{x2} = 0.8357 \mid \alpha = 0.05$

The p-value, 0.9406, is far greater than the significance level of 0.05. We thus have insufficient evidence to reject the null hypothesis, and maintain that the true average height of grass kept at 32.0° C is not significantly less than that of grass kept at 31.6° C.

The grass held at 29.6°C, (Figure 8B) although initially lower in both wet and dry biomass than the controls, showed a notable increase as the experiment progressed, eventually outperforming the controls (Figure 8A) as well as the grass held at 32°C (Figure 8C). Although the general trend for the biomasses at the lowest temperature is upwards, the last data point is an outlier, indicating a slight drop in biomass on the last day of testing. At 32°C, biomass remained relatively consistent, and averaged higher with respect to the dry mass of the roots and the wet weight as compared to the control samples.
Figure 8: The amount of biomass collected from each of the three trays under different temperature conditions, with A) 31.6°C, B) 29.6°C, and C) 32°C

The grass at 32°C possessed much higher absorbance readings in the green wavelength, and thus the most chloroplasts. The grass at 29.6°C possessed the next highest number of chloroplasts, followed by the control.

Figure 9: Absorbance spectrums for chloroplasts in temperature-variant trays with (A) initial values and (B) final values; Tray 1 = Red; Tray 6 = Yellow; Tray 7 = Blue

In the control trays, there was minimal plasmolysis and wilting taking place. However, the higher temperature tray was weak in color and plasmolysis from the beginning of the trial, while the lower temperature tray began to deteriorate in both categories on Day 4.
Table 3: Color and plasmolysis for grass under conditions of a variable temperature

<table>
<thead>
<tr>
<th></th>
<th>Control Trays 1 and 3</th>
<th>Temperature Trays Lower Temperature (29.6°C)</th>
<th>Temperature Trays Higher Temperature (32.0°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day #</td>
<td>Color</td>
<td>Plasmolysis</td>
<td>Day #</td>
</tr>
<tr>
<td>1</td>
<td>Bright Green</td>
<td>No</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Bright Green</td>
<td>No</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Bright Green</td>
<td>No</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>Bright Green</td>
<td>Yes</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>Lighter Green</td>
<td>Yes</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>Lighter Green</td>
<td>Yes</td>
<td>6</td>
</tr>
</tbody>
</table>

In the temperature variant trials, the low temperature trial had the largest rate of dissolved oxygen growth, indicating that lower temperatures are ideal for algae growth.

![Effects of Temperature on Dissolved Oxygen](image)

Figure 10: Dissolved oxygen in algae under temperature-variant conditions

**Water-Variant**

The tray of grass watered with 180.0 mL/day maintained its density the best during the trial, showing little density change in Figure 11. The control and the 120.0 mL tray show a falling trend in density, with the 120.0 mL tray displaying a harsher drop in density over time.
While the height of the control grass shows only slight growth in Figure 12, the tray with 180.0 mL/day shows a significantly larger rate of growth, with the only outlier in height being on Day 2 of measurements. The results for the tray with only 120.0 mL of water per day were in the opposite direction, with the average height generally deteriorating over the six day span.

Statistical Analysis: Control (150 mL daily) versus Increased Water (180 mL daily)

Given that $\mu_1$ is the true mean growth height for the grass treated with 180 mL of water daily and $\mu_2$ is the true mean growth height for the grass treated with 150 mL of water daily, assume that the sample is random, independent, and normal, then run a two-sample T test.

\[ t = 0.4401 \mid p = 0.3356 \mid \bar{x}_1 = 12.212 \mid \bar{x}_2 = 11.920 \mid S_{x1} = 1.3916 \mid S_{x2} = 0.8357 \mid \alpha = 0.05 \]

The p-value, 0.3356, is greater than the significance level of 0.05. We thus have insufficient evidence to reject the null hypothesis, and maintain that the true average height of grass treated with 180 mL of water daily is not significantly greater than that of grass treated with 150 mL of water daily.

Statistical Analysis: Control (150 mL daily) and Decreased Water (120 mL daily)
\( H_0: \mu_1 = \mu_2 \), \( H_a: \mu_1 < \mu_2 \)

Given that \( \mu_1 \) is the true mean growth height for the grass treated with 120 mL of water daily and \( \mu_2 \) is the true mean growth height for the grass treated with 150 mL of water daily, assume that the sample is random, independent, and normal, then run a two-sample T test.

\[
t = -4.0678 \mid p = 0.0023 \mid \bar{x}_1 = 8.670 \mid \bar{x}_2 = 11.920 \mid S_{x1} = 1.7697 \mid S_{x2} = 0.8357 \mid \alpha = 0.05
\]

The p-value, 0.0023, is less than the significance level of 0.05. There is sufficient evidence to reject the null hypothesis, and accept the alternative: the true average height of grass treated with 120 mL/day is significantly less than that of grass treated with 150 mL/day.

The biomass, both wet and dry, for the tray of grass watered with 180.0 mL/day was higher than that of both the control tray and the tray watered with 120.0 mL/day, except for certain results with flaws in the sampling technique, and a miniscule weight was recorded.

![Figure 13: Biomass of grass blades and roots under conditions of a variable amount of daily watering](image)

In both the initial reading and final reading, the control tray of grass possesses lower green absorbance and lower chloroplast count than the two treatment trays. Of the two treatment trays, the tray given 180 mL of water daily possessed higher absorbance, and thus healthier and more numerous chloroplasts, than the tray given 120 mL/day.
Figure 14: Absorbance spectrums for chloroplasts in water-variant trays with (A) initial values and (B) final values; Tray 1 = Red; Tray 9 = Blue; Tray 10 = Yellow

In the control trays, there was minimal plasmolysis/wilting taking place. However, after that Control Tray 1 and Tray 10 (Less Water) exhibited wilting, matting, and more breakage. Tray 9 (More Water) and Control Tray 3 remained strong in color and did not show any signs of plasmolysis.

Table 4: Color and plasmolysis for grass under conditions of a variable amount of daily watering

<table>
<thead>
<tr>
<th>Control Trays 1 and 3</th>
<th>Water Trays More Water (180 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day #</strong></td>
<td><strong>Color</strong></td>
</tr>
<tr>
<td>1</td>
<td>Bright Green</td>
</tr>
<tr>
<td>2</td>
<td>Bright Green</td>
</tr>
<tr>
<td>3</td>
<td>Bright Green</td>
</tr>
<tr>
<td>4</td>
<td>Bright Green</td>
</tr>
<tr>
<td>5</td>
<td>Lighter Green</td>
</tr>
<tr>
<td>6</td>
<td>Lighter Green</td>
</tr>
</tbody>
</table>

The pH stayed constant at a pH of 6.0 for trays 1, 3, and 4 on Day 3. Then, the pH fluctuated between 5.5 to 7.0 for trays 5, 6, 7, 9, and 10 on Day 3. Trays 1, 3, 4, 5, 6, 7, and 9 had a pH of 6.5 on Day 6. The pH of tray 10 on Day 6 was 7.0.

Table 5: Change in pH over the trial period for each tray

<table>
<thead>
<tr>
<th>Tray #</th>
<th>Day 3</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>6.5</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>6.5</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>6.5</td>
</tr>
<tr>
<td>5</td>
<td>6.5</td>
<td>6.5</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>6.5</td>
</tr>
<tr>
<td>7</td>
<td>5.5</td>
<td>6.5</td>
</tr>
<tr>
<td>9</td>
<td>6.5</td>
<td>6.5</td>
</tr>
<tr>
<td>10</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>
DISCUSSION

The purpose of the CO$_2$ variable was to test how increased concentrations of atmospheric CO$_2$ would impact growth. It was hypothesized that increased exposure to CO$_2$ would increase photosynthetic activity and grass growth. Because grass is photosynthetic, it chemically reduces the carbon present in atmospheric CO$_2$ by converting it into usable energy. In addition to producing high photosynthetic rates, increased concentrations of atmospheric CO$_2$ also decreases stomatal conductance of water, optimizing the growth capability by allowing the grass to maintain high photosynthetic rates with comparatively low rates of water loss through the stomata pores. Ultimately, the carbon, oxygen and hydrogen assimilated into organic molecules by photosynthesis constitute approximately 96% of the total dry mass of a typical plant (15). Therefore, it is plausible to conclude that photosynthesis is critical to the grass’ nutritional metabolism; increasing the availability of CO$_2$ for photosynthesis can positively affect growth and aspects of plant physiology.

Consistent with the aforementioned theory and the original hypothesis, the grass tray exposed to the highest amount of CO$_2$ at 850 ppm demonstrated the greatest growth over the 6 days, as can be seen in Figure 2. However, inconsistent with biological theory, the grass tray exposed to 550 ppm CO$_2$ demonstrated a negative trend in grass blade growth over the course of the 6 days. The control tray displayed fairly constant growth throughout the duration of the experiment, exhibiting the least significant growth out of the three grass trays. In fact, the control tray which was exposed to 400 ppm CO$_2$ demonstrated greater growth than the tray of grass exposed to 550 ppm. This can be explained by various factors, such as gratuitous exposure to CO$_2$ and oversaturated soil due to excessive watering of the grass.

The control tray was exposed to 400 ppm CO$_2$ to simulate real-life conditions, since the current atmospheric concentration of CO$_2$ is approximately 400 ppm. As seen in Figure 2, the grass in this tray grew at a slow but steady rate up until day 4, when it reached its peak and actually decreased in growth rate for days 5 and 6. This could be explained by the fact that the grass blades reached their growth limit at day 4 and further exposure to CO$_2$ and water after day 4 actually had deleterious effects on the grass because it was too much. In addition, because more and more grass blades were removed with each passing day, towards the end of the experiment, there was a shortage of soil and roots remaining in the tray to absorb the 150 mL of water that were dutifully added to the trays every day regardless of the amount of soil in the tray. Consequently, the grass blades were heavily flooded in the last two days of the experiment, resulting in decreased grass growth during those two days.

The tray exposed to 850 ppm CO$_2$ was designed to simulate possible real-life conditions in 100 years. The concentration of CO$_2$ in the atmosphere is steadily increasing, and scientists project that by 2100, the CO$_2$ level on earth will have risen to 850 ppm. In the short amount of time during which this experiment was conducted, the grass demonstrated the greatest growth under these conditions. Similarly, the algae exposed to the highest CO$_2$ concentration also had the highest growth rate, because the growth of algae is often limited by the availability of CO$_2$ in the atmosphere. However, in real life, maintaining 850 ppm of CO$_2$ in the atmosphere would have consequences on all natural communities such as the mass melting of ice, rising sea levels, greater precipitation, destruction of natural habitats, and forced changes to various ecosystems.
All in all, while the increased exposure of this grass tray to CO$_2$ was effective in the short term, this result would not be desirable.

It was originally believed that the grass in the 550 ppm tray would demonstrate greater growth than the 400 ppm control tray, but less growth than the 850 ppm tray. However, the 550 ppm tray ultimately had the poorest quality of growth, as shown in Figure 2. For the first 3 days, the grass in this tray grew positively as expected, but after Day 3, the grass began to wither and growth was stunted. This could be explained by similar reasons as the other trays in that after day 3, the grass reached its maximum capability and was thus unable to absorb any more CO$_2$ or nutrients. At this critical turning point, any exposure to additional CO$_2$, water, and sunlight was in fact detrimental to the grass. Thus, rising CO$_2$ levels on Earth may have negative impacts on producers as well as other forms of life. In addition, as can be seen in Figure 1, all three trays demonstrated a decreasing trend in growth density over the 6 days. As the grass was exposed to more water and CO$_2$, less grass grew in the specified area of 32.4 cm$^2$. This further corroborates the assertion that overnutrition actually has pernicious implications upon plant growth.

The objective of the temperature-variant experiment was to observe the effects of temperature change on plant productivity. In the near future, the average global temperature is expected to increase, which is consistent with the experimental temperatures. Ryegrass optimally grows between the temperatures of 15.6°C and 21.1°C, and the ryegrass was grown between 29.6°C and 32°C in this experiment (17). As the results show, the highest growth in density (Figure 6), height (Figure 7), and biomass (Figure 8) was not found in the control tray, but actually in the increased and decreased temperature trays. The particular phenomenon of the distribution of the plant growth can be explained by the fact that ryegrass optimally grows at a lower temperature than was tested in this experiment. This made the decreased temperature tray closest to the optimal temperature and also the most productive. On the other hand, the results demonstrated that the control and decreased temperature had nearly the same number of chloroplasts while the increased temperature had more (shown in Figure 9), thus contradicting the notion that the higher-temperature grass would be less healthy (18). However, the hypothesis was still supported by the trend lines in Figure 7 that showed upward sloping growth rates in the grass height. We had hypothesized that higher temperatures would harm the ryegrass more, but this hypothesis was not completely consistent with the data.

The results proved our hypothesis to be partially incorrect because while the lowest temperature tray was the most productive in terms of blade density, as demonstrated by its positively sloped trendline in Figure 6, the highest temperature tested showed the most growth in terms of grass height averaged out point by point in Figure 7. The high productivity of the lowest temperature can be attributed to its short distance from the optimal ryegrass growing temperature, but the productivity of the highest temperature can be attributed to human error and not optimal growing conditions; theoretically it should have had the lowest growth rate.

Also, in real life conditions, the variables are in effect concurrently. When temperature is increased, usually rainfall is decreased, which would decrease its productivity. When temperature was taken into account for the hypothesis, it was under the conditions that the amount of water it would receive would be held constant from tray to tray. In a real life situation, when ryegrass would be subjected to increased temperature and decreased water, its productivity
would decrease past that of ryegrass growing in lower temperatures (19). Moreover, as the largest contributor to the natural greenhouse effect, water vapour plays an essential role in the Earth’s climate. However, the amount of water vapour in the atmosphere is controlled mostly by air temperature, rather than by emissions. For that reason, scientists consider it a feedback agent rather than a forcing agent to climate change. Therefore, water treatment was considered essential for the present experiment.

In order to compare the effects of an elevated provision of water versus a partial deficit of water, the health traits of each tray were compared to the outcome of the control. These water-variant trays were watered in a manner detailed in Equation 1, and the margins of error inherent in the calculations allow for the rounding off of the figures to the nearest decimeter.

The results of data collection demonstrate that in most cases, the tray watered with 180.0 mL outperformed the control with respect to grass growth, in contrast to our hypothesis. However, the difficulty experienced by the 120.0 mL/day tray in growing into a healthy organism was in line with the prediction made prior to the six-day trial period. These properties are observed in the plots of both average height (Figure 11) and growth density (Figure 12). The biomass results showed that the 120 mL/day tray generally produced the highest biomass, both dry and wet (Figure 13), thus illustrating that the ryegrass responded positively to decreased simulated precipitation. Except for in a few anomalous cases, this observation was valid; in the few instances with smaller mass readings, there was likely large amounts of human error in the washing and weighing of the grass. It is possible that the human technicians washed away or discarded parts of roots and blades, or performed inconsistent washing. In the case of chloroplast count (Figure 14), both treatment trays were clearly superior to the control trays, demonstrating that amounts of precipitation both lower and higher than the average global mean allow for greater chloroplast development (Table 4). As expected, there was no change from Day 3 to Day 6 in pH (Table 5), as the daily watering maintained the pH around a neutral state with no excess CO₂ to make the soil more acidic.

The amount of precipitation for the control was calculated based on the global mean in the present day, and as a result, this value deviates from the optimal amount of water for ryegrass. The ryegrass theoretically would grow best at an annual rainfall of 27”, whereas the global mean was calculated to be 40.7677” (20). However, in contrast to the logical conclusion that an excess of water would be harmful to the development of the ryegrass, the grass flourished under the increased-water conditions of 180.0 mL. This phenomenon likely occurred as a consequence of the non-optimal high temperatures, as the grass required more moisture to survive in the hot environment.

Another possible influential factor was the lack of drainage for the trays. The grass often flooded as a result of water pooling, with no dry soil remaining to absorb the water. In a real-life scenario, the soil would extend further down into the ground, allowing for more absorption. However, a limited amount of soil was present in the trays, so grass growth may have been inhibited by excess water. Additionally, standing water may have interfered with the roots of the ryegrass, thus stunting the growth of the plant. For the best result, then if we had implemented a drainage system, we could have avoided waterlogging of the soil for more realistic observations.
CONCLUSION

As research suggests, global climate change will have noticeable effects on the environment and change the functioning of ecosystems. Changing temperatures, CO\textsubscript{2} levels, and weather patterns will cause plants to adapt to new living conditions, both positive and negative. The agriculture industry will be directly affected by global climate change, since the type of plant species and growing location will be shifted to fit within the parameters of available resources. The results of our experiment have critical implications in the context of future climate change, as global warming will continue to be an issue in our industrialized society.

ACKNOWLEDGEMENTS

We would like to extend our gratitude and appreciation to Dr. Cassano for always being there in times of need, Dr. Srivastava for providing us with invaluable knowledge and support, Runi Patel for being the essential backbone of this project by assisting us at any hour of the day, and last but not least, all NJGSS donors for allowing us to attend this prestigious program.

REFERENCES


11. Average precipitation in depth (mm per year) [Internet]. The World Bank Group; [cited 2014 Jul 29]. Available from: http://data.worldbank.org/indicator/AG.LND.PRCP.MM


[8-20]