Impact of elevated [CO2] on *Brassica rapa* leaf count, biomass, and leaf area.

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Abstract

[CO2] is expected to reach between 600 and 900 ppm before 2100. Increased [CO2] is known to increase photosynthetic carbon fixation. We grew *Brassica rapa* for 13 days in [CO2] 400 and 800 ppm under constant temperature and lighting to investigate the effects of increased [CO2] on leaf count, leaf area, and biomass in *B. rapa*. We found that the mean leaf area, biomass, and leaf count of the control group was higher than the means of the treatment. These results are inconsistent with previous research and knowledge of photosynthetic carbon fixation, so sources of error such as incorrect calibration, planting, and watering, or some other process that would produce such an effect on a group-wide scale were attributed to these results.

Introduction

The UN Intergovernmental Panel on Climate Change (IPCC) predicts atmospheric carbon dioxide concentration to reach 500-600 ppm by 2050, with some models predicting levels as high as 900 ppm by 2100. Investigations into organismal response to predicted rises in CO2 levels are vital in order to reasonably prepare for human adaptation to these drastic environmental changes. Of particular importance is the response of plants, organisms which not only fix inorganic carbon but also support the vast majority of heterotrophic organisms, such as humans. It is critical for humanity to understand and respond the effect of increasing [CO2] on crop plants that humans use to feed their massive population if they are to survive without massive population loss in future climatic conditions. Previous studies have found increased [CO2] promotes plant growth: Omer and Horvath (1989) found greatest biomass increase in response to increased [CO2] at 25°C, while Ackerly et al. (1992) found an increase in leaf initiation rate in response to increased [CO2] around. Kimball (1980) showed a biomass increase of 10-143% in C3 crops when [CO2] was doubled. *Brassica rapa*, an important food crop used all over the world, is one such C3 plant that will be affected by changes in [CO2]. Based on these results, we hypothesized that photosynthetic carbon fixation in *Brassica rapa* plants would increase under increased [CO2]. Under a carbon enriched atmosphere, plants will have excess CO2 for photosynthesis, and because of this will have increased water-use efficiency. With excess atmospheric carbon, plants are able to increase their total stomatal conductance by increasing the number of leaves and and keeping the average stomatal index per leaf the same, or by decreasing the number of leaves but increasing the average stomatal index per leaf, assuming other resources are not a limiting factor.

Methods

We grew two groups (n=12) of turnip seeds at an ambient [CO_2] of 400 ppm and a treatment [CO_2] of 800 ppm over a period of 13 days. Both groups were held at a constant temperature of 25 °C with a 24-hour lightsource for the duration of the experiment. Planting quads were filled ½ full with moist planting media. Media was then tamped down, and three Osmocote fertilizer pellets were planted, after which more media was added and tamped down until the quads were ½ full again. More planting media was added until the soil was 1 cm from the top, then 1 *Brassica rapa* seed was planted in the middle of each quad cell 2-4 mm beneath the surface of the soil. Each set of quads were labelled as “control” or “treatment, and cells were labelled 1-12. Quads were watered twice daily from 9/26 through 9/30 and watered once daily from 10/1 through 10/8. Leaf number, leaf size, biomass and biomass allocation were measured for each plant and averaged. Leaf area was determined by measuring the width and length of the largest leaf on each plant. Biomass was determined by uprooting the plants and separating soil from the roots. The plants were then put in envelopes, and each group was put in a paper bag and dried in an oven at 60 C for ~24 hours. After the plants were dry, each plant was weighed using a scale.

Statistical analysis was conducted via a t-test with a 95% confidence interval. This test was used to determine if variation of leaf count, biomass, and leaf area between the treatment and control groups exceeds that of variation within the groups.

Data analysis

Figure 1: **Results of t-tests for leaf count, leaf area, and plant total dry biomass, and means of treatment and control groups**. Seed #4 in the control and seed #6 in the treatment did not sprout and these data points were not included in the t-tests or averages for any of the metrics. Seeds #9 and #11 in the treatment sprouted late, and were also not included in the t-tests or averages. Seed #7 in the control had an abnormally large biomass, and was excluded from the mean and t-test of biomass only.

	Control mean	Treatment mean	T-value	P-value
Leaf count (leaves)	3.73 (n=11, SE=0.1950)	3.22 (n=9, SE=0.1470)	2.07	0.0542
Leaf area (cm^2)	9.51 (n=11, SE=0.5775)	8.46 (n=9, SE=0.5623)	1.30	0.2095
Biomass (mg)	81.50 (n=10,	68.79 (n=9,	2.45	0.0271

	SE=3.230)	SE=4.063)		

Results and Discussion

The means for the number of leaves, leaf area, and biomass in the control group were larger than those of the treatment group. The biomass of the two groups was the only statistically significant variable ($t=2.45$, $p<0.05$), however leaf count between the two groups was nearly statistically significant ($t=2.07$, $p=0.0542$). Leaf area was not statistically significant between the two groups ($t=1.30$, $p=0.2095$). These results refute our hypothesis, and conflict with the results of previous similar experiments done with different plants. While this result is novel, it is due to extreme scrutiny based on previous research and what is known about photosynthetic carbon fixation in C3 plants, so further research is needed to determine the causation of such a result.

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