Dark Stomatal Response and Carbon Dioxide Levels in *Dudleya lanceolata* at Various Humidities

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Abstract not centered

Crassulacean Acid Metabolism (CAM) is a photosynthetic adaptation carried out by various plants that thrive in arid conditions, such as cacti and succulents. In order to reduce water loss, CAM plants undergo a diurnal cycle of opening their stomata at night and then closing them during the day. This experiment was constructed to determine whether humidity levels, in the absence of light, would affect the carbon dioxide consumption and stomatal response of *Dudleya lanceolata*. The leaves of eight *D. lanceolata* plants were acclimated to four different humidities. Carbon dioxide levels and stomatal imprints were taken. The metabolic rates at each humidity were averaged and no statistical significance was found (p=0.655, ANOVA). Conclusion?

Introduction

There are several methods of carbon fixation a plant can use to convert carbon dioxide into glucose. In C3 plants carbon fixation is initially performed via the enzyme rubisco. Rubisco takes carbon dioxide and adds it to ribulose bisphosphate, initiating the first step of the Calvin Cycle. During the presence of light, these plants open their stomata during the day to allow for gas exchange. Stomata then close at night when light is no longer available (Hartsock & Nobel, 1975). Crassulacean Acid Metabolism (CAM) is a photosynthetic adaptation carried out by various plants that thrive in arid conditions, such as cacti and succulents. In order to reduce water loss, CAM plants undergo a diurnal cycle of opening their stomata at night and then closing them during the day. Since carbon dioxide consumption does not occur during the day, it is stored primarily as malic acid in vacuoles until light is available. Once light is present, carbon dioxide is released and the Calvin cycle begins (Ting, 1985).

In C3 plants, light stimulates potassium ions to enter guard cells causing them to become turgid, as this a hyposmotic effect and causing water to move into the cell; resulting in open stomata and the entrance of carbon dioxide into the cell. On the other hand, CAM plants have an endogenous “photoperiodic circadian rhythm” (is this a cited quote or a commonly used phrase?) meaning they perform a 24-hour daily cycle in which they open and close their stomata with the influence of external cues, the presence/ absence of light and water (Lee 2010). There are numerous plant species that exhibit the ability to “switch” from CAM to C3 or take on characteristics from both methods of carbon fixation. Plants that possess the ability to switch from CAM to C3 are termed facultative CAM plants and can open and close their stomata either during the day or night. This switch in carbon fixation is dependent on various environmental factors such as availability and salinity of water, temperature, humidity, or photoperiod (Sayed, 2001; Cushman, 2001 and Lange & Medina, 1979). It is known that several species of *Dudleya* are facultative CAM plants (Nobel & Brutta, 2007). This experiment was constructed to determine whether humidity levels, in the absence of light, would affect the carbon dioxide consumption and stomatal response of the facultative CAM plant *Dudleya lanceolata*. (Might want to give your postulations why you think this may or may not occur…might help the reader understand your desire to conduct this experiment in the first place.)

Materials and Methods

Eight small *Dudleya lanceolata* plants were purchased from Tree of Life Nursery (San Juan Capistrano, CA). The plants, all similar in size, were placed outside in direct sunlight during the day and
then moved inside at dusk to prevent freezing. They were watered every other day in the evening, at seven pm, with 25 mL of deionized water.

Trials began on March 24, 2010 and continued through April 4, 2010. The testing was conducted at the house of Amanda Swanson (Laguna Hills, CA). Located on site were four Pasco GLX data loggers with carbon dioxide probes and photosynthesis chambers provided by Saddleback College Department of Biology. A photosynthesis chamber is a two chambered tank in which the center, inner portion can be sealed off, via a rubber stopper, to create a sealed, isolated environment.

The four probes were set up and calibrated, prior to each testing, to verify that all equipment was functioning properly.

At the beginning of the experiment, each plant was given a number for identification and a control was established to ensure that the plants were not performing carbon fixation. The control was set up during the daylight at ambient temperature and humidity (35%). For the control, a leaf from each plant was removed and placed into the photosynthesis tank and the carbon dioxide levels were measured.

The appropriate saturated solution for each humidity was determined using tables that indicated which solution (at a temperature between 20 and 25°C) would create which humidity in a closed system; tables from different sources were referenced to ensure accuracy (Greenspan, 1976; Sweetman, 1933; Winston & Bates, 1960). The saturated solutions were then prepared. The 0% humidity environment was produced by placing 15 mL of drierite in the bottom center portion of the photosynthesis chamber to absorb all the moisture. A rubber stopper was placed in the middle of the drierite so that the leaf was not directly in contact with the drierite to prevent direct contact with the leaf. To produce a 33% humidity environment 15 mL of saturated MgCl was placed at the bottom portion of the chamber. The 75% humidity environment had 15 mL of a saturated NaCl solution. The 100% humidity level was obtained by placing 15mL of deionized water in the center portion of the tank. Each humidity condition had a rubber stopper in the tank so that the leaves could rest in the chamber without contamination or damage from the solutions.

Each leaf was removed from the plant to eliminate soil-water potential from directly affecting stomatal response during the acclimation period through data collection. One 7 cm length of leaf was removed by cutting with scissors. The cut portion of the plant was covered with parafilm to prevent any potential water loss. With no light present, the leaves with the parafilm were weighed and then placed into the center portion of the appropriate photosynthesis chamber. Two leaves, from separate plants, were placed in a photosynthesis chamber to ensure that sufficient CO₂ levels could be detected. The leaves were paired consistently. After the leaves were allowed to acclimate for three hours at the respective environment condition, the Pasco data logger CO₂ probes were turned on to record data for an additional three hours.

Once the CO₂ data were collected, the leaves were removed from the photosynthesis tanks and immediately reweighed. Stomatal imprints were then obtained by first adding a drop of Superglue to a blank glass slide. The top of the leaf was then firmly pressed into the wet glue and held there for 10 seconds before being slowly pulled off. The glue was given time to dry and then later analyzed under a compound light microscope magnified at 100x. The trials were repeated with different freshly cut leaves and parafilm for four trials to ensure that each plant was rotated through every humidity level. Stomatal data were analyzed by photographing the stomata and counting the number of open stomata compared to the total number of stomata visible (an area of 1 mm² was visible). Statistical analyses were run using Microsoft Excel; all data were analyzed by converting parts per million (ppm) of CO₂ to grams of CO₂ produced per gram of plant.

**Results**

The metabolic rate of the leaves at each humidity level was averaged and graphed (Figure 1). There was no significant statistical difference between plant metabolic rate and humidity level (p=0.655,
ANOVA). At 0% humidity, the average metabolic rate was $7.25 \times 10^{-7} \pm 4.18 \times 10^{-7}$; at 33% humidity, average metabolic rate was $1.20 \times 10^{-6} \pm 8.62 \times 10^{-7}$; at 75% humidity, average metabolic rate was $9.50 \times 10^{-7} \pm 1.43 \times 10^{-6}$; at 100% humidity, average metabolic rate was $1.58 \times 10^{-6} \pm 2.59 \times 10^{-6}$. Stomatal imprints indicated that 44% of the stomata were open for 0% humidity, 41% open for 33% humidity, 50% were open for 75% humidity and 54% of the stomata were open for 100% humidity (Figure 2).

Figure 1. The mean metabolic rates for each humidity. ANOVA shows no significant difference between humidities ($p=0.655$). Error bars indicate mean ± SEM.

Figure 2. Mean percentages of stomata opened for each humidity.

Discussion

The results of this study do not support the hypothesis since there was no significant difference between CO$_2$ levels or stomatal response and humidity. However, other research suggests that humidity does in fact play a role in stomatal response; however, and there are mixed results regarding the effect of humidity on CO$_2$ consumption (Lange and Medina, 1979; Griffiths et al., 1986; Luttge, U. et al., 1986). A study done by Herppich (1997) proposed that stomata do in fact respond to humidity, but that this stomatal reaction was not absolutely linked to CO$_2$ consumption at night in Plectranthus marrubioides. The research showed that drought stress played a large role in the plant’s ability to fixate carbon. When well watered, there was no link between CO$_2$ uptake with stomatal response and humidity levels. However, in extreme drought situations, humidity levels did affect CO$_2$ consumption (Herppich, 1997).
Guard Cell Turgidity

Stomatal opening is greatly influenced by turgidity within the guard cells; the more turgid the guard cells, the more open the stomata will be. Turgidity is determined by the amount of ions either entering or leaving the cell (MacRobbie, 2006). The movement of these ions follows an osmotic gradient and in order for the guard cells to become turgid, the cell must also take up water from its surroundings. The influx of water and ions into the guard cell vacuole creates pressure and the stomata then opens (Sheriff & Meidner, 1975). Since/because the leaves were removed from the plants, Therefore it is likely that there may have been a decrease in the overall water content within each leaf over the six hour period. Upon weighing the leaves after the six hours, there did appear to be a decrease in the weight of each leaf. If this weight loss was in fact water loss, then the guard cell vacuoles may have been prevented from gaining enough osmotic pressure to become turgid and to fully open the stomata.

Acclimation

Although the leaves were allowed to acclimate for several hours, it is possible that this acclimation time was not sufficient. CAM plants are extremely adaptive to their environments and it has been shown that upon the introduction of an environmental change, it takes a longer period of time for them to show any significant change in their physiological behavior (Szarek, et al. 1987; Hartsock & Nobel, 1976). In several studies, the acclimation time in which the plants were exposed to experimental changes was a minimum of two weeks. The independent variables in these studies were CO$_2$ levels (Szarek et al., 1987) and water availability (Hartsock & Nobel, 1976). Theise leads to the possibility that the amount of time allowed for the leaves to acclimate may have been insufficient. (you already made the same statement in your first sentence)

Leaf Age

Another interesting factor that may have contributed to this study was the age of the leaves. A study done by Jones (1974) showed that leaf age contributed to CO$_2$ exchange in Bryophyllum fedtschenkoi, another CAM plant. Young leaves did not display CAM and displayed CO$_2$ output during the night. Mature leaves on the other hand, did perform CAM. They suspected that the older leaves may have had more vacuole space and were able to store higher quantities of CO$_2$ as a result. Although the leaves used from D. lancolata were all the same length, it is possible that there was variation in leaf age and maturity level.

Literature Cited


According to the citations book they wanted us to use, you may need to restructure your citations to this format:

Last name, First Name (or Initial), Middle Initial (if any), subsequent authors (if any). Year. Article title. *Publication*, volume number(issue number):inclusive pages.

Might want to double check with Steve on that.
Author(s): Jennifer A. Oberholtzer and Amanda C. Swanson

Title: Dark Stomatal Response and Carbon Dioxide Levels in *Dudleya lanceolata* at Various Humidities

**Summary**

Summarize the paper succinctly and dispassionately. Do not criticize here, just show that you understood the paper.

Researchers were interested in studying the effects of various humidities on the stomatal response and carbon dioxide consumption of facultative CAM plant leaves. By utilizing Pasco GLX data loggers with carbon dioxide probes and photosynthesis chambers, the researchers placed paired leaves, covered in parafilm, into the chambers and recorded their CO$_2$ output. Afterwards, a stomatal imprint of each leaf’s surface was created using superglue, then the number of open to closed stomata quantified per sq mm. Results conclude that there was no statistical difference between CO$_2$ production and humidity level, and the number of stomata’s open varied per sq mm as the humidity levels rose. Researchers account for this result by arguing the following: (1) because each leaf was removed from the host plant, the leaf’s guard cells did not have enough osmotic pressure created by water uptake to open properly; (2) plants were insufficiently acclimatized to the various humidity levels prior to conducting the data collection; and (3) the leaves use may have been differing ages, making their overall CO$_2$ consumption abilities vary.

**General Comments**

Generally explain the paper’s strengths and weaknesses and whether they are serious, or important to our current state of knowledge.

The research was well-thought through and overall carefully administrated. The discussions of why the results were not significant were also nicely researched.

Due to research time constraints it is understood why more trials could not be conducted, though the increase in data may have helped bring the SEMs down a bit.

Your introduction should have some postulations of why you think humidity affects CO$_2$ production and stomatal response. Interested readers should be able to see the scientific implications behind CO$_2$ consumption and increase in humidity, particularly with regards to today's ever-changing climates (though if this is one of the researchers motivations behind the study, you may want to add that to the Introduction as well), thus the study’s scientific relevance is necessary to understand climatic effects on gaseous exchange and photosynthesis. And, though not terribly critical, it would be helpful if you could add *Dudleya lanceolata*’s common name to the Introduction.
Technical Criticism
Review technical issues, organization and clarity. Provide a table of typographical errors, grammatical errors, and minor textual problems. **It's not the reviewer's job to copy Edit the paper, mark the manuscript.**

☐ This paper was a final version  ■ This paper was a rough draft

Manuscript marked with reviewer comments.

Recommendation

☐ This paper should be published as is  ■ This paper should be published with revision  ☐ This paper should not be published

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