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Stomatal Conductance Trends of the Jade Plant (*Crassula ovata*) in Relation to Circadian Rhythm Entrainability

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ABSTRACT

The effect of an inverse light cycle on a Crassulacean acid metabolism (CAM) plant was observed in the study. CAM plants are unique in that they open their stomata at night in order to conserve water, an evolution that has come about because these plants primarily exist in very arid climates. By placing a plant in a chamber in which the lights could be programmed to turn on when it was dark out, and to turn off during normal daylight hours, the stomatal conductance of the leaves of a jade plant (*Crassula ovata*) were recorded several times a day over a five-day period. The results were analyzed in comparison to a control jade plant that was found on the campus of Pepperdine University. The study was mostly inconclusive, due to the fact that the control plant was outside in the rain, while the experimental plant was in dry conditions in the chamber. In addition, the experimental plant did not show a strong correlation to the expected results, which could be because it was only treated for a few days.

INTRODUCTION

Various mechanistic tendencies of plants has been explained by the concept of a biological clock. This biological clock we talk of exists within the plants as a circadian rhythm. Plants endogenously measure time through consistent environmental cues that present themselves everyday and every night. Three fundamental parameters guide a plant's circadian rhythm: periodicity, entrainability, and temperature compensation (McClung 2001). The natural 24 hour day and night transitions of earth is the periodicity most plants, animals, and us humans abide by. Mechanisms that follow a circadian rhythm include movement and growth, gas exchange, CO₂ assimilation, and hormone productivity and responsiveness.

Crassulacean acid metabolism (CAM) plants are interesting in that their stomata open during the night, and close during the day, unlike traditional C₃ and C₄ plants (Osmond 1981). This is due to the plants adaption to arid environments, in which CO₂ is collected at night instead of in the daytime to reduce evapotranspiration. The CO₂ is fixated by phosphoenolpyruvate carboxylase (PEP carboxylase) and stored in vacuoles, and is then released into the chloroplasts during the day when the stomata are closed to activate the Calvin Cycle. It is often argued whether this temporal division of labor is dictated through a circadian rhythm, or by simple light and dark transitions. The temporal division of labor stems from the CAM plants' desire to optimize stomatal conductance at night compared to the day. We hypothesize that manipulating sunlight and darkness, the primary environmental cues dictating circadian rhythm, will reset the CAM plant's biological clock, therefore revealing the entrainability of the plant's stomatal conductance habits. Specifically, we are testing the adaptation capabilities of the Jade Plant (*Crassula ovata*), which are prevalent on the Pepperdine Campus located in Malibu, CA. This will be quantified through comparisons of measurements in stomatal conductance of a tested species and the predicted trend.

HYPOTHESES

H₁: A CAM Jade Plant (*Crassula ovata*) transplanted into a reverse 24-hour light system will be able to adapt and follow our predicted stomatal conductance trend, confirming the reset of its circadian rhythm.

H₀: A CAM Jade Plant (*Crassula ovata*) transplanted into a reverse 24-hour light system will not be able to adapt and follow our predicted stomatal conductance trend.

PROCEDURE



SUBJECT: The tested Jade Plant was already pre-potted by Dr. Stephen Davis. Six leaves of the plant were chosen at random (from separate branches) and flagged and numbered as the leaves used for testing.

INSTRUMENTS: The Jade Plant was placed in a BioChambers Inc. Model GC-20 to manipulate the light and dark perceived by the plant, creating a transplanted system experiment. In a reversal of actual 24 hour day, we set the darkness from 7:00 AM - 5:00 PM, and lightness from 5:00 PM - 7:00 AM. The temperature was also slightly adjusted, measuring 22°C at times of darkness, and 24°C at times of light.

PROCEDURE: Measurements were taken using a steady-state porometer (Decagon Devices; Model SC-1). This calculated stomatal conductance in the units mmol/m²s. Measurements were taken over a five-day span, at consistent times of the day corresponding to the manipulated system's sunrise (5:00PM), sunset (7:00AM), and middle of the night (12:00PM).

RESULTS

Figure A: Stomatal Conductance @ 7:00AM

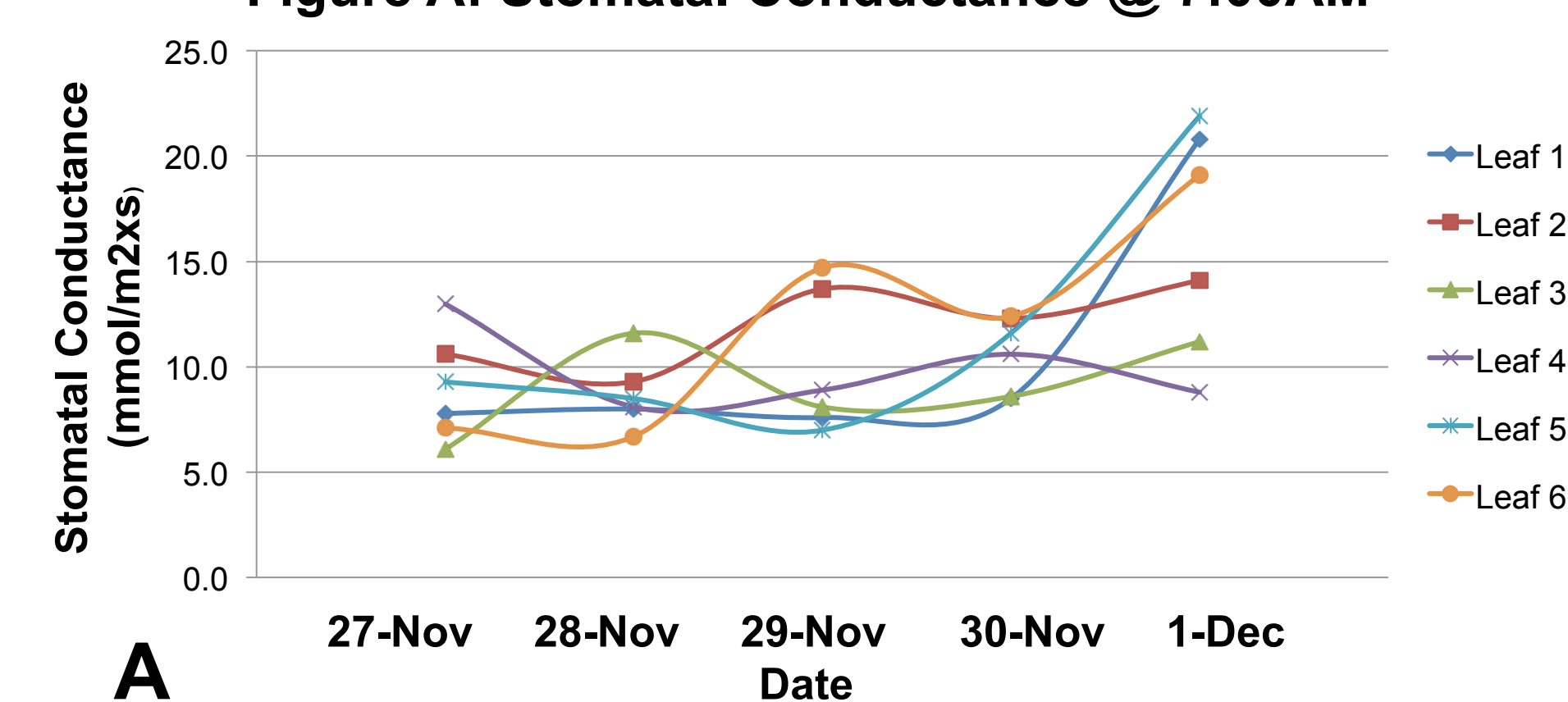


Figure B: Stomatal Conductance @ 12:00PM

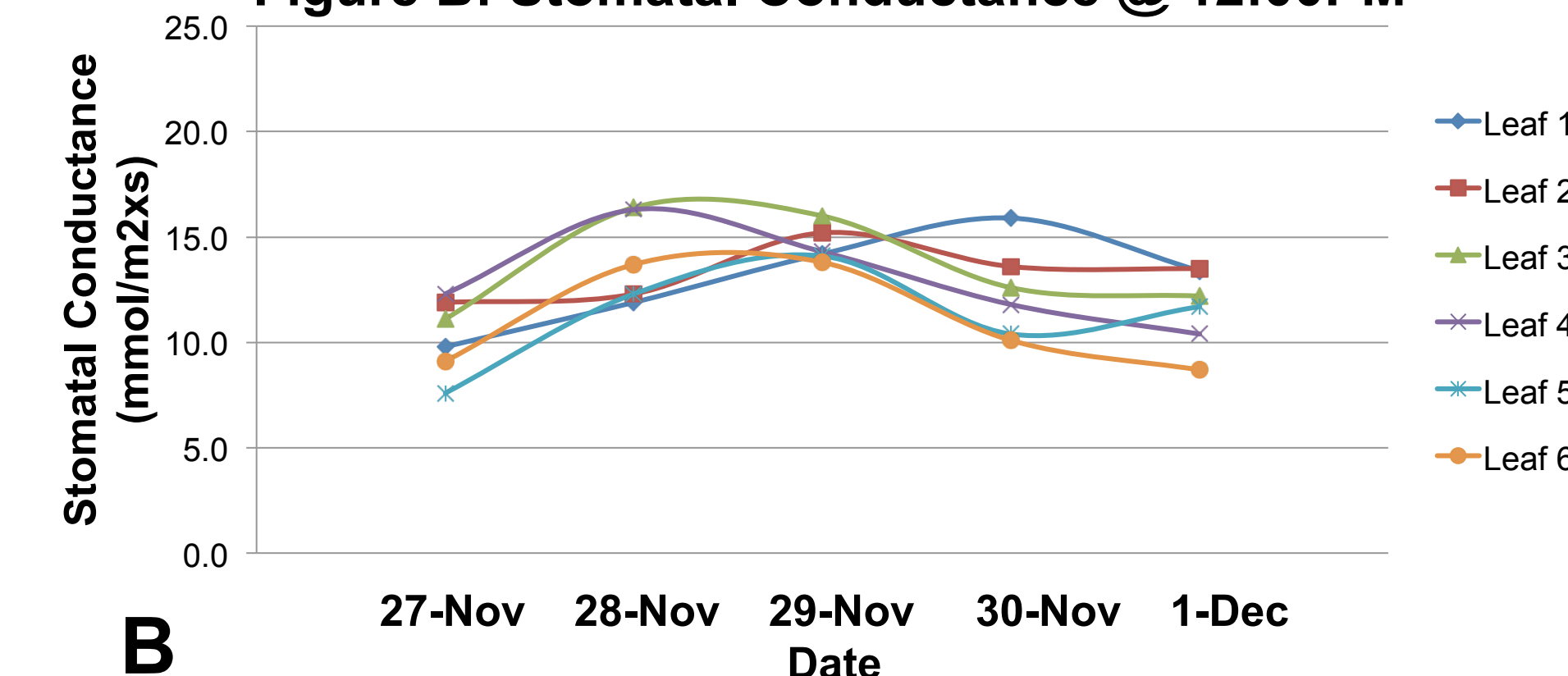


Figure C: Stomatal Conductance @ 5:00PM

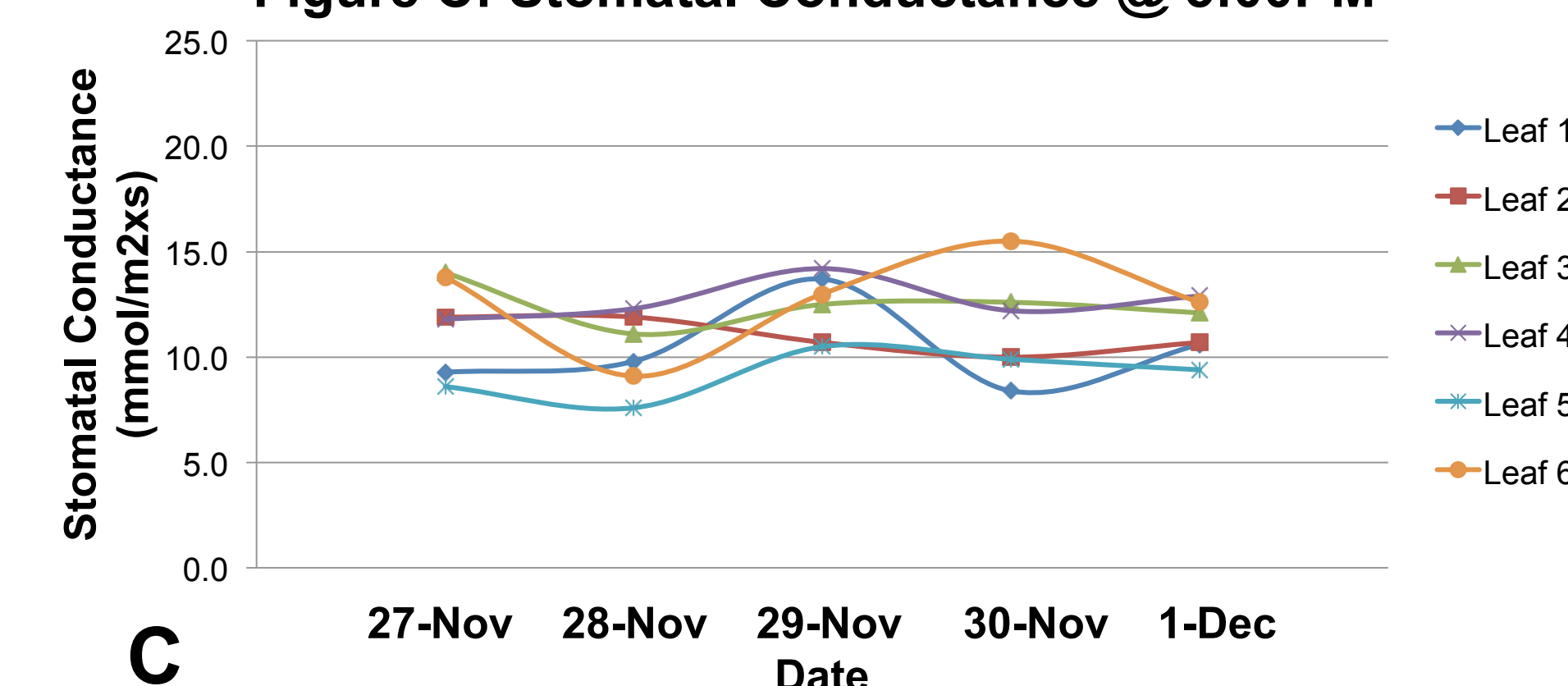


Figure D: Average Stomatal Conductance @ 7:00AM

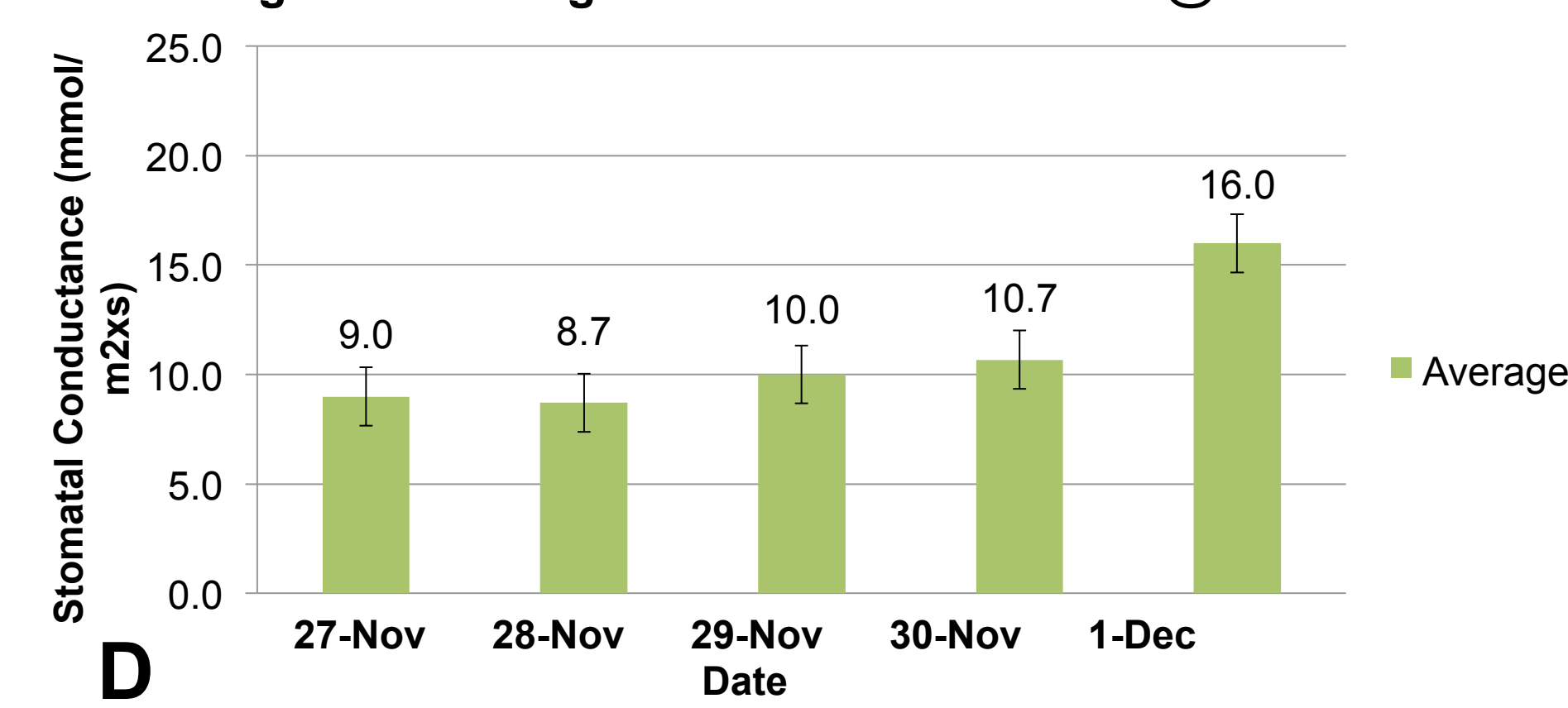


Figure E: Average Stomatal Conductance @ 12:00PM

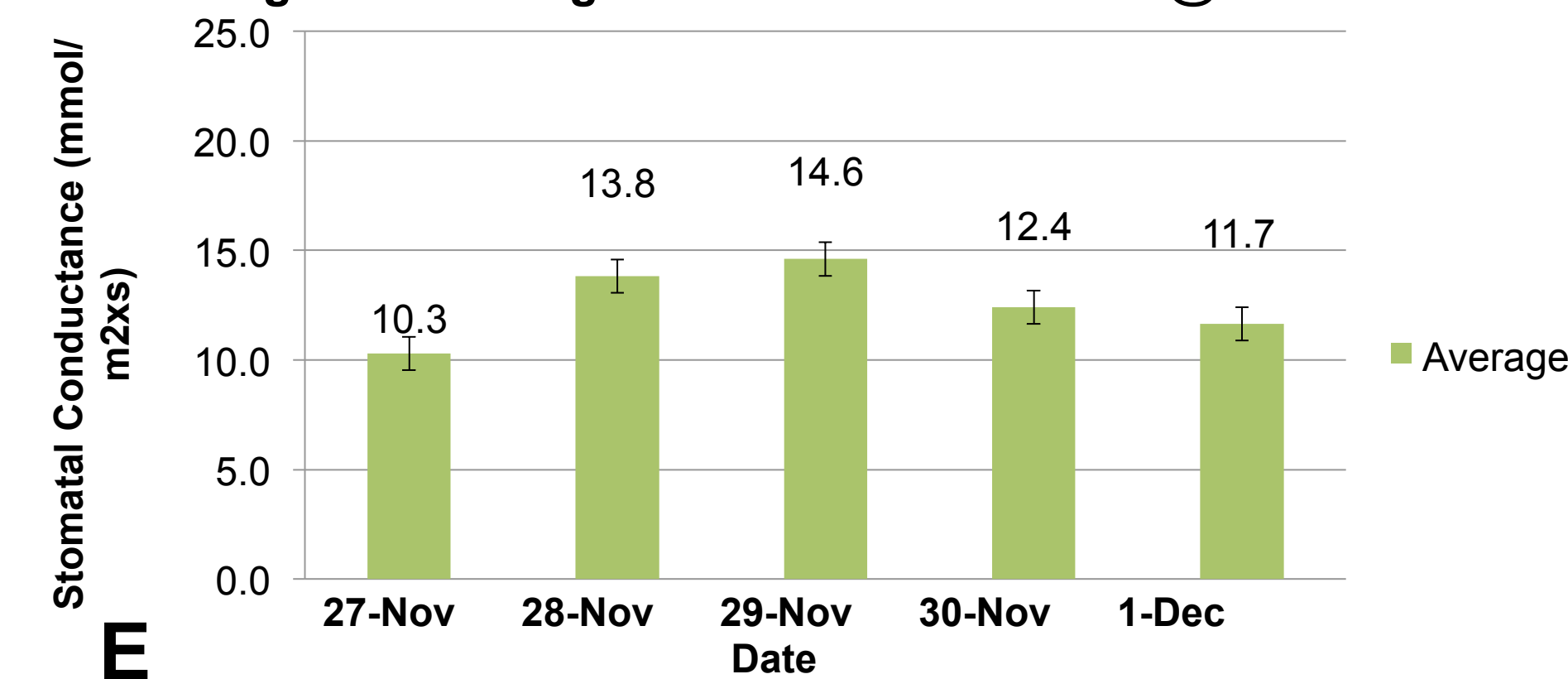
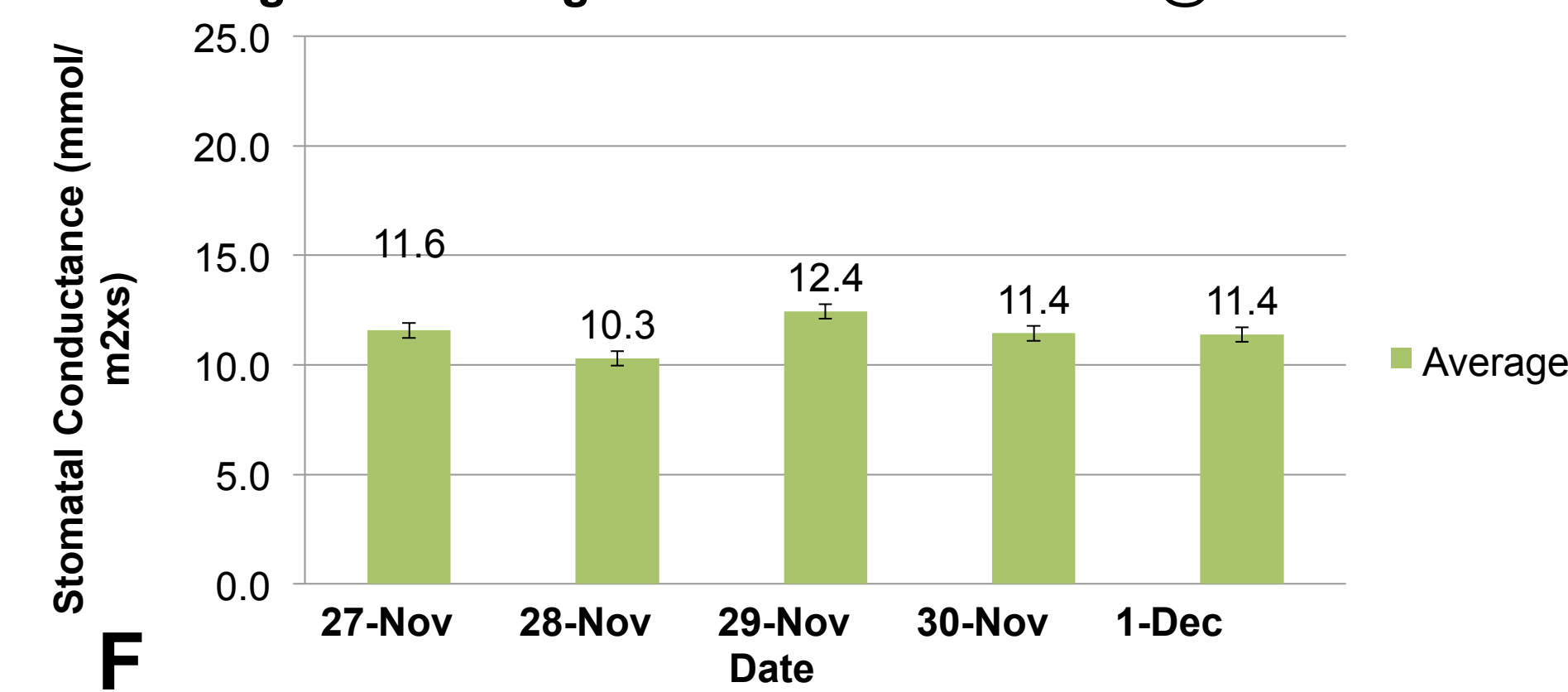


Figure F: Average Stomatal Conductance @ 5:00PM



Stomatal Conductance was plotted over time to show trends for individual leaves (Figures A-C) and the average conductance for the plant (Figures D-F) at varying times during the day. Unfortunately the trends did not follow the expected results, which would have had an increase in conductance for all three times of day as the experiment progressed and the CAM plant became entrained to a new environment.

DISCUSSION

Based on the results, we cannot accept our hypothesis. It appears that the pores were most open during the middle of the day, and were more closed both in the morning and night. Despite this being accurate with our predicted trend, there was not enough of a significant change from morning to nighttime to accept our hypothesis. We have determined several ways to hopefully improve our results the next time we run a similar procedure.

One source of potential error was simply not running the procedure for an adequate amount of time. As the sample plant was only treated for a five-day period, it may not have had enough time with the different light cycle to adjust. It appeared as the week went on that stomatal conductance during normal daylight hours increased, which led to the conclusion that the procedure should have been run longer. In addition, we failed to take readings of stomatal conductance at night. In order to truly test to see if our treatment was taking effect, the stomata should have been checked during normal nighttime hours. If there was a significant difference and they were closed, as we hypothesized, this would demonstrate with more clarity that our treatment was at least somewhat effective. In addition, anytime measurements were taken the chamber had to be opened, which allowed light to enter into the chamber and hit the plant. Although it was a very minimal amount, doing this several times a day may have altered the opening of the stomata slightly and skewed the results. Furthermore, it was difficult to get a true reading of the control plant. Unfortunately, during the week of the procedure, the weather in Malibu was not conducive to collecting results due to an abnormal amount of rainfall. Because our test plant was kept in a dry, controlled chamber, and the untreated plants were basically in constant mist for the entire week, our measurements were incompatible. Next time, it would be best to check the forecast before running the experiment, or to run a control plant in a chamber with lights set to mimic a normal day.

If this procedure were to be performed again, it would be advisable to use more subjects (in order to create more data), as well as keep the plants under the treatment for a longer time. Plants that have had a set circadian rhythm for their entire life will not quickly get out of that rhythm. Also, a better control must be set up so that the experimental data can be accurately compared to a plant under normal conditions. If these steps are carefully followed, it is certainly possible that the results could be significant and it could be shown that plants can adapt their circadian rhythm to a different set of environmental cues.

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