Effect of Light Intensity on *Dionaea muscipula* Photosynthesis Measured by Action Potential Response Time to Close Trap

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Abstract

Photosynthesis particularities such as light intensity and the coupled action potential exhibited visually in a dramatic fashion by *Dionaea muscipula* is of investigative value. This plant's electrophysiology in the absence of a true nervous system can provide insights into understanding how environmental changes affect all plants in general. We hypothesized that light being a reactant of photosynthesis, greater intensity will lead to greater photosynthetic rate which we were able to quantify by proxy of the action potential response time of Venus flytrap closing. This was done by means of video recording in slow motion and appropriate software analysis tools. The plants were kept in a stable environment with no external light interference or major temperature fluctuations and positioned under LED grow lights set at different light intensities resulting in five treatment conditions with three independent samples. Although statistical analysis by one-way ANOVA suggests no significant difference between the mean response time of the traps closing in the different lighting conditions hence failing to reject our null hypothesis, a suggestive trend was observed with faster response of the plant traps in brighter lighting conditions as was initially predicted and supported by literature. Interestingly, the fastest response time was found to be in the 80% condition which would be around the 6000lux mark leading us to suspect that this is an optimal lighting condition for plants with needs alike *D. muscipula*'s. At greater light intensities the chlorophyll complex pigments are in higher proportion leading to higher photosynthetic rates (Nelson and Cox) and more pronounced response of the electrical cascade of reactions that generate the plant closing its trap event (Volkov et al.; Trebacz et al.). Nonetheless, our result was not statistically significant and further studies with suggested improvements would need to be conducted to be able to arrive to more confident conclusions and determine the effect of light intensity on the photosynthetic rate of *D. muscipula.*

A. Introduction

It is widely known that photosynthesis is a vital process in autotrophs like plants in which light energy is used to convert water and carbon dioxide into organic matter for the plants' growth. The light energy is captured by a light-harvesting-chlorophyll protein complex which are made up of among other things, the pigments chlorophyll a and b (Nelson and Cox). Most interestingly however, the ratio of pigments which control the functioning of proteins during photosynthesis is modulated by light intensity, or rather the difference between actual lighting conditions and ideal conditions (Biswal et al.). Since light is a reactant of photosynthesis, this suggests that more intense light will lead to a greater photosynthetic rate. This is where *Dionaea muscipula*, or the Venus flytrap comes in thanks to its carnivorous acquired tastes hypothesized to have evolved from root material in order to harvest nutrients from air (Stokstad); it has been called numerous times a marvel biochemically, electrically and mechanically (Volkov et al.; Forterre et al.; Ueda and Nakamura) due to its capability to shut its trap to immobilize prey under 0.5 seconds (Ueda et al.) – a response triggered by an all or none action potential alike animal neurotransmission which is highly variable according to its environment, be it light or temperature for example. Hence, the previously mentioned photosynthetic rate can be connected to and easily measured by examining the plant trap closing upon mechanical stimulation of its hair receptor cells that are meant to capture the movement of a fly enclosed in the trap and send electrical signals to close the said trap (Pavlovič et al.). Markedly, this is of particular interest providing insights into understanding how environmental changes affect plants in general. Light spectrum or light intensity changes, nutrient availability, temperature, and humidity are all direct or indirect effects of climate change (Higuchi and Hisamatsu) and investigating these environmental changes affecting agricultural practices would help us solve challenges head on and come up with creative and sustainable solutions.

Given that *Dionaea muscipula,* or Venus flytrap exhibits dramatically any environmental changes and is this highly selective especially since operating the trap takes considerable amount of energy and trade-offs (Lehtinen), it must act most readily when conditions are favourable i.e., optimal light intensity. We expect that with higher light intensity, also considering that the plant's habitat is full sun wet savannahs (Missouri Botanical Garden), more chlorophyll pigments will be activated leading to a greater photosynthetic rate exhibited by a faster closing of the trap. It is important to point out that optimal intensity of light for plant growth and photosynthesis of full-sun-requiring plants has been previously investigated to be around 6000lux (Nguyen et al.), therefore in the higher light intensity closest to this quantity we expect to observe traps closing the fastest.

We will examine the effect of light intensity on Venus flytrap's photosynthetic rate by proxy of the response time of the action potential signal closing the plant trap. Hence, the null hypothesis H_o states that increasing the light intensity will have no effect on the plant's response time among the different lighting conditions. On the other hand, the alternative hypothesis H_A states that increasing the light intensity will indeed decrease the response time in *D. muscipula* indicating a better photosynthetic rate compared to other lighting conditions.

By investigating lighting condition behaviour of plants, we hoped to give an interesting insight in plant physiology in the modern times of greenhouses, apartment balcony vertical gardens, and futuristic projects like terraforming where knowing physiological processes related to molecule fluxes and ion channel activities in the plant vascular system will help in building plant growth models that are efficient and nutrient rich (Higuchi and Hisamatsu).

B. Methods

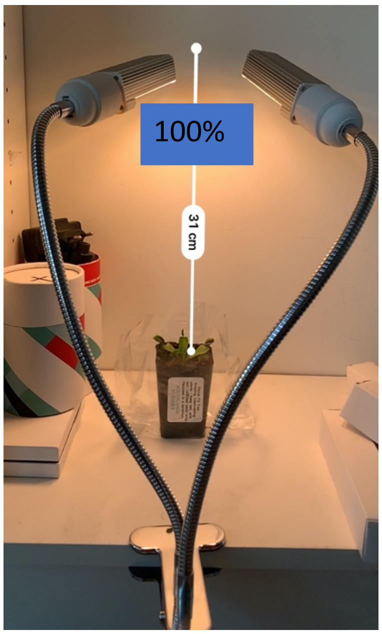

Figure 1: Example of plant and lamp setup. Flytrap directly under lamp bar light direction, here intensity set at 100% or 7100 lux at 12-inch (or around 31cm) distance from light source. Plant left in cellophane wrapping to keep some humidity in its environment.

This experiment looked at *Dionaea muscipula*'s response to a mechanical poke that would elicit the plant's trap closing under five different light intensity conditions as follows: 100% which would correspond to 7100 lux at a 12-inch distance from the LED lamp bar, 80% (5700 lux), 60% (4300 lux), 40% (2850 lux), and 20% (1450 lux), achieved using purchased plant growth LED lights with adjustable light intensity that claim 96ppfd (or 7100 lux) at 12-inch distance when set at full intensity. The LED lights were also adjusted to turn on and turn off at the same time each day on a twelve-hour schedule of lighting. We obtained the *D. muscipula* plants from a local (Vancouver) plant nursery. In each condition a Venus flytrap nursery pot with at least three viable traps per pot which were the replicates was set directly under the lamp bar on separate shelves (refer to Figure 1 for setup example) with the cellophane wrapping left on to conserve some humidity. The plants were set in a room with minimum external light and temperature fluctuation interferences. External light was removed by keeping the blinds down in the room and temperature was recorded for each testing day to account for potential extraneous variables. A phone camera in slow motion was used to record the trap closing upon touching the hair cell with a wooden skewer to mimic a fly touching the trap and the response time or the time it took for a trap to close after a certain number of pokes which were also noted in observations. This recording was done for each treatment condition for a total of two weeks of data collection. The plants were watered with distilled water in a spray bottle every other two days to keep the soil moist and humidity constant as flytraps are native to wetlands (Missouri Botanical Garden) and require that. The videos were analyzed using "Tracker" software to be able to approximate the response time up to at least hundredths second by setting the frame per second speed to 120 or 240 as appropriate and setting the time to zero just before the trap movement is initiated and pausing when the plant response is ending. Finally, the results were statistically evaluated by a one-way

ANOVA test, performed on the mean response times of all five treatment groups to determine F-ratio and p-value to 95% confidence interval.

C. Results

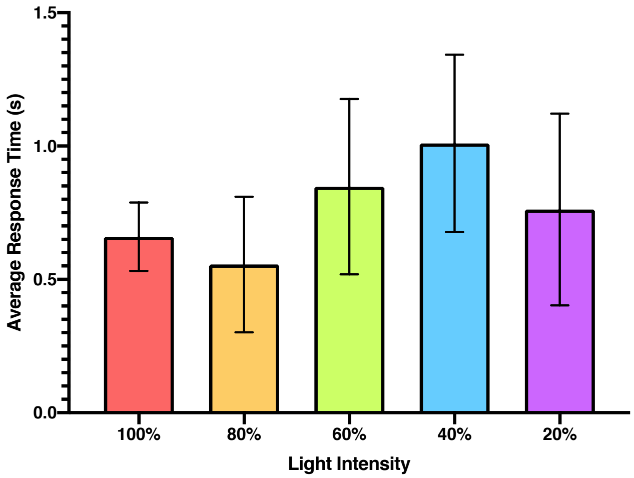

Figure 2: Average response time in seconds of Venus flytrap closing under 5 different light intensity conditions (100% corresponding to 7100 lux, 80% to 5700 lux, 60% to 4300 lux, 40% to 2850 lux, and 20% to 1450 lux). Response time was averaged between the three replicates in each condition. Error bars represent the standard deviation in appropriate size per each condition and specifically in respective order of appearance on the figure (Average response time ± error bar size in seconds) 0.7±0.1 s (n=5), 0.6±0.3 s (n=12), 0.8±0.3 s (n=7), 1.0±0.3 s (n=5), and 0.8±0.4 s (n=12), where n is the number of days successful data was collected for each condition respectively. Statistical analysis one-way ANOVA yielded p-value of 0.0654.

Response time measurements were carried out by means of video recording in slow motion later analyzed in detail (refer to Methods) using "Tracker" software yielding up to 3 decimal precision. Throughout the experiment no major morphological changes were observed except for the last 2 days of the experiment the traps of the 100% lighting condition plant seemed to turn yellow/red indicating of possible light scald. To control for possible confounding variables, we took temperature measurements for each day of the experiment for a total of 12 days fluctuating between 20.7°C to 23.2°C, averaging at 22±1°C; the higher temperatures occurred when the thermostat in the apartment was turned higher due to colder weather, however that was curbed by keeping a window open for the remainder of the experiment leading to a relatively constant room temperature around 21°C. One-way ANOVA test was performed using "Prism" software and appropriate conclusions were made in discussion given the obtained p-value of 0.0654. Figure 2 shows a trend of lower response time, meaning faster trap closing in the higher light intensity conditions, specifically lowest at 80%. The highest response time was at 40% light intensity out of the lower lighting conditions. It is important to note that the response time in the 80% condition is nearly half the time observed in the lower lighting of 40%. As the light intensity decreases the response time increases generally, with the exception of the 20% condition leveling with the 60% lighting condition. This trend of response time is congruent with the initial expectations as noted in the discussion despite the large error bars/standard deviation.

D. Discussion

We examined the effect of changing light intensity on photosynthetic rate of *D. muscipula* by proxy of the action potential response time in closing the plant trap upon mechanical stimulation of the trigger hairs found inside the trap. Our one-way ANOVA statistical analysis showed no statistically significant difference in the mean response time between the lighting conditions, with a p-value bigger than the 95% confidence interval, thus we failed to reject the null hypothesis and any observed differences from Figure 2 were then attributed to random chance. In other words, higher light intensity did not have any effect on the plants' response time.

Nonetheless, a trend was clearly observed in Figure 2, appearing that at the higher light intensities (100% and 80% that is) the flytraps closed much faster than the lower ranges of light intensities. Specifically, the 80% condition (or 5700 lux) exhibited the fastest response time which as mentioned beforehand is around the optimal light intensity for photosynthesis of full-sun-requiring plants alike *D. muscipula*, only 300 lux shy of the 6000 threshold (Nguyen et al.). It is noteworthy that we observed a slight increase in response time at the 100% lighting condition, combined with the fact that we observed towards the end of the experiment possible signs of light scald/burns to the traps of the plant, which could be due to absorption of excessive light energy by the photosynthetic system leading to impairment of the light-harvesting-chlorophyll protein complex reactions in plant cells (Nelson and Cox; Tang et al.).

The trend of increasing response time with decreasing light intensity correlates with the initial predictions as well as the current literature (Biswal et al.; Feng et al.; Zhang et al.) where it was found there is increased chlorophyll a and b production in the increased lighting conditions, a model also supported mathematically (Peeters and Eilers) where higher light intensity closely correlates with higher photosynthetic rate. Moreover to explain the proxy we used in this experiment, the faster response rate of the plant to mechanical manipulation, assuming that *D. muscipula* cannot discriminate between the simple mechanical triggering we did in this experiment or wounding of the trigger hairs when an insect is trapped (Pavlovič et al.), was due to the fact that with increased light intensity, chlorophyll pigments become increasingly more activated triggering a cascade of reactions in the photosynthetic system of plants (Nelson and Cox). Consequently, as part of the cascade of photosynthetic reactions, a membrane pump keeps cytosolic concentration of Calcium ions low, and the better the photosynthetic rate the more efficient this process is (Nelson and Cox; Trebacz et al.). An action potential, in this case the trigger in trap closing, in fact increases the cytosolic concentration of Calcium free ions (Trebacz et al.) meaning that

Calcium ions rush into the cytosol to generate an action potential which is a sort of communication in vascular plant systems (Nelson and Cox), and so at higher photosynthetic rates the gradient will be greater as more Calcium ions will be initially out of the cytosol due to the aforementioned more efficient pump. Hence, with a higher photosynthetic rate due to higher light intensity the action potential response time in closing *D. muscipula'*s trap becomes faster.

However, slight variations from this expected trend, namely the 20% condition results that are slightly incongruent with this model by exhibiting response time of comparable value to the 60% condition could be attributed to experimental errors and uncertainties further discussed.

In an effort to minimize extraneous variables we made sure to keep experiment room without any external light interference by keeping the blinds down in the room and not switching on any other light sources. This was mostly successful apart from a few times beyond our control when the room had to be illuminated for other considerations, which could explain the high variation in the treatment conditions, but mostly for the 20% condition with a standard deviation of half the mean response. Given that the 20% condition plant was set on a shelf closest to the room light fixture and considering that any light interferences would be most evident in the lowest lighting, hence the large variation that we observed (refer to Figure 2). Unfortunately, we were not able to write down in the observations the days when this error occurred (which would help in identifying outliers) as it was beyond our control or knowledge until much later.

On the other hand, temperature measurements were taken for each testing day to account for any possible variations that temperature could confound as that is another factor influencing *D. muscipula*'s trap closing (Volkov et al.). We were able to control room temperature with only small variations which upon closer inspection of the raw data did not lead to any discernible differences (refer to Results). These type of controls for extraneous variables allowed us to demonstrate a clear trend as previously discussed that is congruent with our initial expectations.

To reiterate, the observed differences however not statistically significant, cannot be ignored when an evident trend in Figure 2 is observed. The high variations shown by the error bars, even accounting for the previously mentioned uncertainties like external light and temperature, possibly would have normalized if the experiment were to be carried on for longer. Even though we collected data for twelve days in total, this was variable between the conditions, as there were times when some traps refused to close or even to open after a day as the off chance of catching a fruit fly presented itself a few times, or conditions of stress were inflicted such as the light scald on the 100% condition trap leaves, or the mechanical "wounding" that is a consequence of this experiment's methods. If one were to repeat this experiment one would need a longer data collection period and possibly more plant pots per condition to serve as replicates rather than number of traps per pot. Albeit the number of replicate pots in this experiment could have been increased, however Venus flytraps are in high demand and a rare find in most nurseries during the season when we purchased our subjects. All in all, if these two adjustments and a closer control of interferences were to be made if one were to repeat this experiment, the data would be closer to be normally distributed and better or more confident conclusions whether rejecting or failing to reject the null hypothesis could be made.

Finally, an application of investigations as such on light intensity and plant physiology and subsequent better understanding of ideal conditions for plant growth, could be better greenhouse systems or even a not so far-fetched project of vertical urban agriculture with increasing urbanization and population growth. LED plant grow lights have been used in the growth of highly vigorous plants such as tomatoes, cucumbers, and roses (Higuchi and Hisamatsu), however studies point that supplemental LED upward lighting in a highly dense cultivation space significantly increases photosynthetic rate and hence growth of the crops (Zhang et al.). With the ability to control lighting conditions like intensity, wavelength, or circadian timing among other plant physiology factors, next generation urban agriculture will be able to support increasingly urbanized global communities more efficiently.

E. Conclusion

We failed to reject the null hypothesis that increasing light intensity will decrease the mean response time of the action potential to close the traps of *Dionaea muscipula.* However, it is noteworthy that we did observe a trend congruent with our initial predictions with response time being at its lowest in alighting condition close to 6000 lux which is a possible optimal light intensity, the brighter lighting condition resulted in slightly longer response time, while the lower lighting conditions showed much slower response time.

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