The Effect of Temperature on the Trap Closure of *Dionaea Muscipula* (Venus Flytrap)

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Abstract

Carnivorous plants have evolved to consume prey items to compensate for the lack of nutrients in their natural habitats. Most species reside in areas where soils are waterlogged and slightly acidified due to fluctuating temperature conditions through the year (Ellison et al., 2008). This makes it difficult to obtain vital nutrients needed for survival. *D. muscipula* is a species of carnivorous plants that trap insects within its leaves using sensitive "trigger" hairs. After trapping the insect, the leaf forms a seal around the insect to process the digestion. For our experiment, we tested three different temperature conditions (0° C, 12° C, 25° C) to measure the speed of trap closure in the Venus flytrap. The closing mechanism was recorded using a slow motion camera and was analyzed over a video editing software to get the precise time it took the trap to close under the given temperature. Previous findings have shown an upwards trend in time with lowering temperatures (Volkov et al., 2008). Thus, we hypothesized that we would find a difference in time it takes for the Venus flytrap to close its traps under different temperature treatments, with the warmer temperature yielding the fastest closure and lowest temperature yielding the slowest trap closure time. Using a one-way ANOVA test, the variation between the three temperature treatments was found to be statistically significant (p-value < 0.01, alpha= 0.05). Our findings were consistent with the previous research and we rejected the null hypothesis. The trap closure time was found to be the fastest in the warmer conditions, in our case the 25°C treatment.

Introduction

The Venus flytrap (*Dionaea Muscipula)* has an active trap closing mechanism that is considered to be one of the most rapid movements in the entire plant kingdom (Volkov et al., 2008). All plants typically have the ability to sense and recognize mechanical stresses that come from wind, or changes in the soil pH. These different kinds of stresses the plant is put under are recognized by its mechanosensory channels (Benolken et al.,1970). The mechanical response for the Venus flytrap *(Dionaea Muscipula)* is quite different from other carnivorous plants. The Venus Flytrap who have such a fast, rapid, and highly noticeable touching stimulus response such as the Venus flytrap (Hodick et al., 1989).

The plant typically grows 5-7 mouth-shaped leaves that are divided into an upper and a lower leaf. The upper leaf consists of trapezoidal lobes that contain three sensitive trigger hairs and red anthocyanin to lure insects into its trap (Volkov et al., 2008). These trigger hairs are lined with hair-like projections called cilia that serve as teeth which form an interlocking cage when the trap is sprung to be closed. These trigger hairs with the addition of cilia are what allow the Venus flytrap to trap unsuspecting prey for consumption. The sizes of these traps typically range anywhere from 3 to 7cm. Touching the trigger hairs on the upper leaf of the plant activates the mechanosensory ion channels which carries out electrical signals within the plant that signals the rapid trap closing mechanism within 0.3 seconds. Upon its closure, the cilia that protrude outwards from the end of each lobe form an interlocking wall that is impenetrable to most insects. The trap does not completely close when these trigger hairs are touched, but the struggling movement of prey within the trap and against these hairs signals the trap to close tighter until a complete seal is formed for digestion (Volkov et al., 2008). This ensures that the energy expended by the plant in closing the trap on prey that has escaped or any other foreign objects is not wasted.

For our experiment, we are proposing a new way to approach and measure how different temperature treatments would affect the kinetics of the trap closure in the Venus flytrap. We hypothesize that if plants have reduced metabolism and cells get damaged at lower temperatures, then we would expect that the time taken for the trap-closing mechanism of *D. muscipula* is increased in colder conditions. The purpose of this experiment is to analyze how temperature would influence the rate of trap closure in *Dionaea Muscipula.*

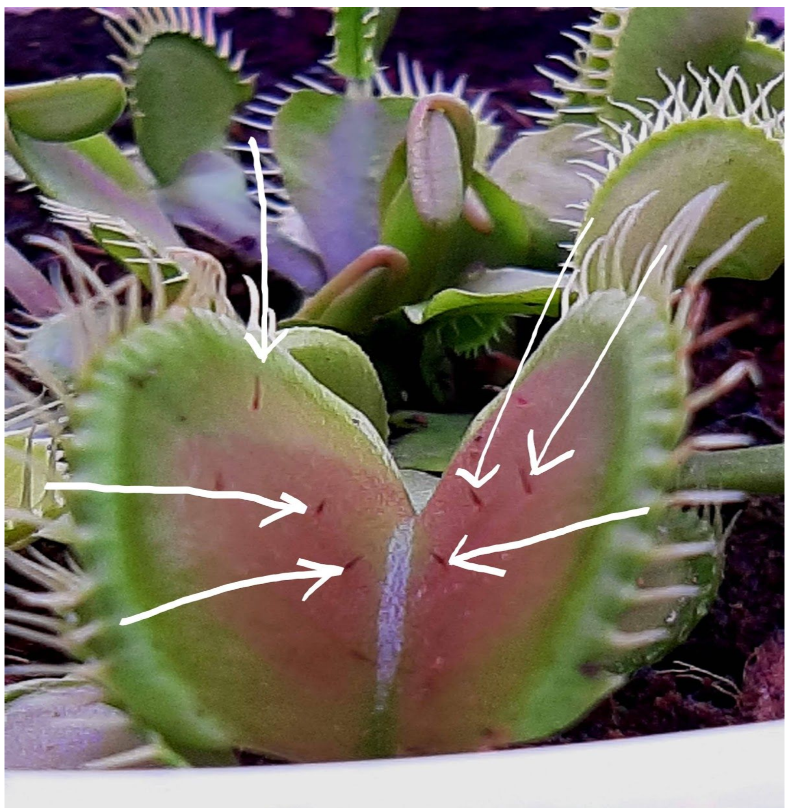

Figure 1: Trigger Hairs, as indicated by white arrows on the Venus Flytrap from the Camera View

Methods

To begin the experiment, we placed our captured pillbugs (prey items) inside of the fridge before feeding. This allowed for a slower locomotion of the pillbugs when placed into the plant's trap and slowed down their kinetics. Next, we placed three Venus flytrap plants into their targeted temperature treatments at 0° C, 12° C, 25° C. The 0° C treatment was placed into a basin with ice and cold water, the 12° C was placed outside under a covered patio, and the 25°C was placed in a room on the windowsill. The temperature readings were ensured and checked by placing a thermometer into the medium (long fiber sphagnum moss) for each of the plants. We then recorded the temperatures after waiting for 5 minutes with the plant in their respective treatments. Later, we prepared our cameras for the slow-motion mode at 240 frames per second (fps) and placed the lens so that the entire trap, trigger hairs, and both lobes could be seen. Then, the recording was started and using a set of tweezers, the prey item (pillbug) were brushed along the trigger hairs of the trap to activate the mechanosensory channels within the plant. We then dropped the pillbug, moving the tweezer aside, and let the plant go through its closing mechanism. The recording was stopped after ensuring that the trap is completely closed and was saved for later analysis. This same process was repeated five times for each one of the temperature treatments for a total of five replicates for each treatment to ensure maximum accuracy of our results.

For the analysis of the collected recordings, we uploaded our clips onto a video editing software, VLC Media Player. For each of the clips two frame numbers were noted. First, the frame number when the 2nd trigger hair was touched by the prey item. Second, the frame number when the trap is fully closed. We then subtracted the first frame number gathered with the second frame number and divided by the frame rate of the camera, 240 fps, in our case. This resulting value gave us the time it took in seconds for the traps to close. We then tabulated this collected data into a data table, and performed statistical analysis. We used a one-way ANOVA test to determine if our recorded results were statistically significant or not.

Results

Three Venus Flytrap plants were placed in different temperature treatments. The temperature treatments were recorded at 0 °C, 12 °C, and 25 °C using a digital thermometer.

Sample #	**0 °C**	**12 °C**	**25 °C**
1	1. 7 s	0.9 s	0.6 s
2	1.6 s	1.0 s	0.5 s
3	1.7 s	0.8 s	0.7 s
4	1.3 s	1.0 s	0.6 s
5	1.5 s	0.9 s	0.7 s
Mean	1.56 s	0.92 s	0.62 s

Table 1: Speed of Closure of Traps at Given Temperature Treatments (seconds)

The mean times taken for the trap to close were 1.56 s at 0 °C, 0.92 s at 12 °C, and 0.62 s at 25 °C. These times were calculated by using the methods in the data analysis section. The video analysis software used was the VLC Media Player and the frame rate of the slow-motion mode on the camera was 240 fps.

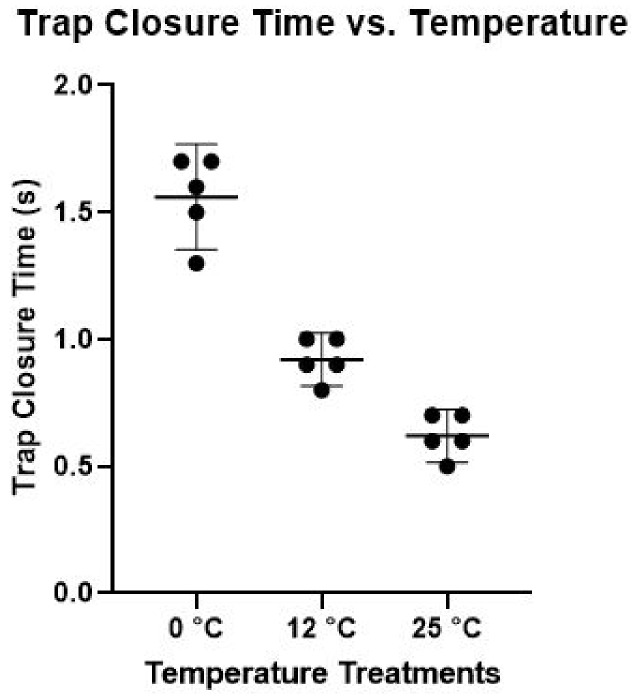

Figure 2: Points represent the times taken for traps to close. Vertical bars represent 95% confidence intervals. Horizontal bars represent the mean volume for a given temperature treatment. There were statistically significant differences between group means as determined by one-way ANOVA ($F(2,12) = 82.33$, $p < 0.0001$).

Discussion

We proceed to reject the null hypothesis ($p < 0.05$) and conclude that our results are statistically significant. That is, the Venus Flytrap plants kept at the three different temperature treatments had different mean trap closure times. We used visual analysis to roughly determine when the traps began to start closing and when they finished closing. This could have contributed to more errors compared to studies using higher grade equipment such as accelerometers to measure the speed of closure. Furthermore, the three plants used are different in terms of maturity and the number of traps each one has, which could have influenced the speeds of trap closure. As we did not account for the maturity of the plant being a potential factor influencing the speed of trap closure, this may have distorted our results. Along with the maturity of the plant, it was unsure as to whether the plant would lose energy for each subsequent trap being closed. Each plant had five traps used for replicates and so if the plant lost a significant amount of energy after each previous trap was closed, the results would be distorted even further because we would expect that a plant with less energy would have traps that close slower. In the study done by Volkov et al., it was mentioned that both electrically and mechanically stimulated Venus Flytraps close in 0.3 s. The fastest mean time observed in our experiment at 25 °C was 0.62 s. We believe that these errors and issues with the experimental design could have created the gap between these values.

Because our results were statistically significant, we know that temperature does affect trap closure time. This time taken for a trap to close can affect the ability of the Venus Flytrap to capture prey items. The traps close slower at lower temperatures, giving prey more time to escape and the result is wasted energy for the plant. As the plants have evolved to supplement the nutrients that are missing from their native waterlogged, acidic conditions by consuming prey, these lower temperatures would have a negative impact on the plant. Venus Flytraps are native to North Carolina in Wilmington where temperatures range from 3 °C lows to 32 °C highs.

Conclusion

Together, these findings suggest that temperature does influence the rate of trap closure in *D.Muscipula.* At the colder temperature (0 °C) treatment the average time for trap closure was found to be the slowest at 1.56 seconds, being the slowest out of the three temperature treatments. This finding is consistent with our original hypothesis that the time taken for the trap-closing mechanism of *D. muscipula* would be increased in colder conditions. Reducing temperature slows down the kinetics within the plant resulting in an overall slower reaction by these carnivorous plants directly impacting the rate of its trap closure. Thus, our findings convey that the rate of trap closure in *D.muscipula* is directly impacted by the temperature it is put in.

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Literature Cited

Benolken, M., Jacobson, S. (1970). Response properties of a sensory hair excised from Venus's flytrap. *The Journal of general physiology*, *56*(1), 64-82.

Bohm, J. (2016). The Venus Flytrap Dionaea muscipula Counts Prey-Induced Action Potentials to Induce Sodium Uptake. *Current Biology*, *26*(3), 286-295.

Ellison, A. (2006). Nutrient Limitation and Stoichiometry of Carnivorous Plants. *Plant Biology*, *8*(6), 740-747.

Hodick, D., Sievers, A. (1989). On the mechanism of trap closure of Venus flytrap (Dionaea muscipula Ellis). *Spring Link*, *179*(1), 32-42.

Pennisi, E. (2020). How Venus flytraps evolved their taste for meat. *Science Mag*, *23*(3), 1-8.

Volkov, A., Adesina, T., Markin, V. (2008). Kinetics and Mechanism of Dionaea muscipula Trap Closing. *Plant Physiology,*, *146*(2), 694-702.

Appendix

Materials and Reagents:

- Venus flytrap (*Dionaea muscipula*) plants - 3
- Pill bugs (*Armadillidiidae spp.*)
- Tweezers
- Thermometer
- Water
- Ice
- Containers
- Camera