

Fall 12-15-2013

Prey Capture in Response to Removing Cilia from Venus Flytraps

Emily Marchini
Coastal Carolina University

Follow this and additional works at: <https://digitalcommons.coastal.edu/honors-theses>

 Part of the [Biology Commons](#)

Recommended Citation

Marchini, Emily, "Prey Capture in Response to Removing Cilia from Venus Flytraps" (2013). *Honors Theses*. 32.
<https://digitalcommons.coastal.edu/honors-theses/32>

This Thesis is brought to you for free and open access by the Honors College and Center for Interdisciplinary Studies at CCU Digital Commons. It has been accepted for inclusion in Honors Theses by an authorized administrator of CCU Digital Commons. For more information, please contact commons@coastal.edu.

Prey Capture in Response to Removing Cilia from Venus Flytraps
2013

BY

Emily Marchini

Biology

Submitted in Partial Fulfillment of the
Requirements for the Degree of Bachelor of Science
In the Honors Program at
Coastal Carolina University

December 2013

Emily Marchini

Prey Capture in Response to Removing Cilia from Venus Flytraps

Introduction:

Carnivorous plants are able to attract, trap, and digest insects. Different types of traps target different prey. There are sticky traps, pitfall traps, and active steel traps (Gibson, 1991). Although this may seem to be a rare modification it appears that there are six origins of carnivorous plants in different groups of angiosperms (Albert et al., 1992). These results indicate that not only were there multiple origins of carnivorous plants, there were also multiple origins of the same trapping mechanism; for example, the flypaper trap has five origins and the pitcher trap has three (Albert et al., 1992). Multiple origins indicate that there were similar environmental factors that caused these plants to evolve carnivory.

Most carnivorous plants are found in well-lit, low-nutrient, moist habitats (Givnish et al., 1984). Ellison (2006) believes that carnivorous plants had to make a choice, resulting in their adaptation to low nutrient environments, even though it put their competitive ability and photosynthetic rates at risk. Carnivory should evolve if the benefits exceed the initial cost of creating the carnivorous modification. The cost of being in a shady and dry environment would exceed the benefit of being carnivorous, which is why carnivorous plants are seldom found in those environments (Ellison and Gotelli, 2001). In a study by Jaffe (1973), traps that were held in dark conditions closed three times slower than those that were held under light conditions. It is clear that light is a very important factor in optimal growth and benefits carnivorous plants. Increasing mineral and nutrient intake could benefit carnivorous plants by potentially increasing overall rates of photosynthesis and seed production (Givnish et al., 1984). Carnivorous plants are

able to acquire nitrogen, potassium, and phosphorous from the insects they trap (Adamec, 2002). They also seem to be able to recycle their nutrients more efficiently than noncarnivorous plants, which is important because of the nutrient-poor environment in which they live (Adamec, 2002). The carnivorous plant that I will be studying is *Dionaea muscipula*, the Venus flytrap.

The Venus flytrap is found in North and South Carolina. The fact that the species is found locally makes it an optimal choice for my study. In its native habitat, the plant depends on a combination of fire, soil moisture, and vegetation (Luken, 2007). The Venus flytrap depends on fire to rid the area of other plant species so more sunlight is available (Roberts and Oosting, 1958). The Venus flytrap is very different from the other 600 species of carnivorous plants. Most carnivorous plants have some sort of attractant, a flower or fragrance, to entice insects to the carnivorous portion of the plant (Ellison and Gotelli, 2001). Most of the insects that were caught by the traps of Venus flytraps were carrying some sort of food, which makes it unlikely it needs to have an attractant to lure in its prey as other carnivorous plants do (Lichtner and Williams, 1977). Another reason the Venus flytrap is different from other carnivorous plants is because it is one of two species that has a trap that is created from modified leaf blades (Hutchens and Luken, 2009). This modified leaf blade is able to snap shut to capture prey.

Carnivorous plants do not directly compete with non-carnivorous plants (Ellison, 2006). The surrounding plants could benefit carnivorous plants by attracting insects that are preyed upon by the Venus flytrap. In order for the insects to get to their intended target, they could travel across the Venus flytrap and become captured. This classifies the Venus flytrap as a filter feeder, because it can seize only prey that happens to walk onto the trap (Jeschke et al., 2004). There are two types of filter feeders, active and passive. Trap builders, such as the Venus flytrap, are considered passive filter feeders (Jeschke et al., 2004).

There are three phases involved in prey capture by the Venus flytrap. The first phase is slow, requiring sensitive trigger hairs to be pressed (Volkov et al., 2011). Insects that are crawling along the face of the trap can stimulate the hairs, and these hairs usually will not be triggered by non-insect material. Temperature also affects trap closure rate, the trap responds rapidly at high temperatures, such as 35°C and 40°C (Brown and Sharp, 1910). Moisture does not seem to have as great of an impact on trap closure as temperature does, in Darwin's studies, he was unable to get the traps to close when he dropped water on the hairs. He was also not able to stimulate the traps to close when he blew on the trigger hairs as hard as he could (Darwin, 1888). This indicates that the Venus flytrap has evolved to adapt to environmental conditions and will not close if there is not any chance for nutrients to be acquired. The trap will not close unless multiple trigger hairs are pushed within a certain amount of time. The trap becomes desensitized to stimuli for 0.75 seconds after one hair is pressed down (Brown and Sharp, 1910). If an insect touches another trigger hair within this time, the trap will not close (Brown and Sharp, 1910). Stimulating the trigger hairs leads to the next phase of trapping.

The second phase is very quick. The snapping shut of the trap requires ATP, although the mechanism involved in producing the rapid motion is not fully understood (Jaffe, 1973). The trap snaps in 0.3 seconds, making it difficult for the prey to escape (Volkov et al., 2011). The trap does not close completely; instead it remains slightly open, with the cilia interlocked. This phase is when the geometry of the trap changes the most (Forterre et al., 2005).

The final stage takes several minutes for the trap to close completely, known as the locking phase (Volkov et al., 2011). Once it locks, there are two paths the plant can take. If prey was not caught, the leaves will reopen within a day (Volkov et al. 2011). If prey was caught, then the leaves will change from convex to concave, crushing the prey item inside (Volkov et al.,

2011). When the trap is completely flattened, a digestive fluid is secreted. The trigger hairs may need to be stimulated again by the struggling prey in order for the fluid to be secreted, which was demonstrated in a study where a pill bug was struggling to escape and continued to cause action potentials in the plant two hours after the trap completely sealed (Affolter and Olivo, 1975). However, additional stimulation is not always necessary for the fluid to be secreted.

The digestive fluid contains proteins, proteinase, phosphatase, DNase, and trace amounts of amylase (Scala et al. 1969). Almost all of these substances are at their maximum concentrations about three days after the plant has locked and flattened its prey (Scala et al., 1969). Each substance plays an important role in the digestion of the prey. For example, the phosphatase is able to break down ATP in the insect, which would make it harder for the prey to escape. The concentration of the substances decreases starting four days after feeding (Scala et al., 1969). The decreased concentration of the substances signals to the plant that the prey has been digested and that the trap can reopen, which may take up to seven days (Volkov et al., 2011). Once reopened, the trap can again catch prey, but it can close only close two or three times before it withers (Jaffe, 1973).

Reopening has several stages that occur over many hours. Reopening is a coordinated event requiring the absence of food as a stimulus (Fagerberger and Howe, 1996). The first stage of reopening is the “sealed phase”. In this phase, the cilia are interlocked and there is a small bulge near the midrib of the trap called the digestion pocket (Fagerberger and Howe, 1996). The next phase is “deappression”, where the digestion pocket begins to flatten out and the bulge moves out toward the lips of the trap (Fagerberger and Howe, 1996). The third phase is “release,” when the lips of the trap are separated but the cilia are still interlocked, resembling a trap that is about to lock (Fagerberger and Howe, 1996). This phase is quick, occurring one to

three hours after the bulge in the deappression stage formed (Fagerberger and Howe, 1996). The final stage is “fully opened,” where the lobes are completely separated, but the trap rarely returns to the same conformation it had prior to closing (Fagerberger and Howe, 1996). This phase takes longer, about ten to twenty hours after the release stage (Fagerberger and Howe, 1996). The complexity of the stages of closing and reopening could be a reason why so many scientists have been fascinated by this plant.

Darwin was one of the first scientists to take interest in the Venus flytrap and he experimented with this plant to see what is required for it to close. He even tried to determine what it could digest; he tested meats and cheeses along with inorganic material (Darwin, 1888). His experiments determined that if the sample was small, the digestive fluid was able to dissolve albumen and gelatin, but not fat (Darwin, 1888). He hypothesized that the traps selected their prey based on size, which was why it took several minutes for the trap to completely shut. By having the trap remain slightly open, the smaller insects would be able to escape (Darwin, 1888). He thought that due to the energy it takes to close the trap and digest prey, small prey would be too costly to collect (Darwin, 1888). He also said that his son witnessed a small insect escaping from between the cilia (Darwin, 1888). To my knowledge, he did not witness this himself, nor did he test it. Not many studies have been done to try to test Darwin’s hypothesis. One study found that in several trap size classes, most of the prey items were ants and spiders (Hutchens and Luken, 2009). So regardless of the size of the trap, there wasn’t a large difference in the size of the prey.

There is not enough evidence to support Darwin’s hypothesis, and I hypothesize that the Venus flytrap collects its prey indiscriminately. It seems that the plant would waste a great amount of energy closing its trap only to let a prey item escape, regardless of size of the prey.

The trap is very sensitive to the amount of force a prey item exerts, and it will not close if the force is weak (Brown and Sharp, 1910). If the insect is big enough to cause an action potential, it should be big enough for the plant to digest. Once the trap is closed, it has to remain closed for up to a day. There could be other prey items that happen to walk on top of the closed trap during that time period. So if the small prey item was allowed to escape, the plant would have wasted several feeding opportunities and received no nutrients. In my experiment, I will remove the cilia from Venus flytraps, which I hypothesize will not have an effect on the overall prey capture rate of the plant or length or prey captured. It has been suggested that the traps have evolved red coloration because the target prey could be “red-blind”, camouflaging the trap in a green environment (Jürgens et al., 2009). The cilia could function as camouflaging the trap in the environment as well.

This experiment has the potential to have a great impact on our understanding of the Venus flytrap’s behavior and the function of cilia on the traps. If we find that the Venus flytrap captures and digests prey indiscriminately, our results would contradict the popular notion that this species selects prey based on size. Furthermore, no one has experimentally manipulated the plants by removing cilia to see how doing so affects their rate of prey capture. If the plants select prey based on size, my first hypothesis would not be supported, and instead this study would provide experimental data to support Darwin’s hypothesis.

Materials and Methods:

In order to test if the procedure would be effective, lab experiments were conducted. This included cutting cilia off traps of different lengths and observing how long it took for the

traps to reopen. Those that were not cut were brushed with scissors in order to determine whether the act of touching alone caused closing or whether the cilia needed to be cut in order for this to occur; some traps closed while others remained open. The traps were given a few days to reopen, and then ants were added to the enclosures. This tested the trapping success of the traps without cilia.

Two field experiments were conducted in Lewis Ocean Bay Heritage Preserve in Horry County, SC. In the first experiment, half of the traps on each plant were cut, the other half were stimulated with scissors. The traps that were closed one week later were removed and placed in 70% ethanol for preserving. This was repeated two times, for a total of three collections. Overall there were 11 individual plants each with 6 traps.

In the second experiment, all the traps on an individual plant were cut or stimulated with scissors. Each experimental individual, the cut individuals, were paired based on trap numbers to a control individual. The traps that were closed one week later were removed and preserved in 70% ethanol. This was repeated two times for a total of three collections. Twenty individual plants were required for this experiment.

The traps were measured, length and width, in millimeters with a caliper. Traps were opened to observe any prey caught. If there was not any prey items, traps were placed in a vial containing 70% ethanol and a label. If there was prey, the length of the prey was measured in millimeters and identified based on its family. The trap and insect were placed in a vial with 70% ethanol and a label. T-tests were used to see if there was a difference in the length of prey captured in cut and uncut traps. Chi-square tests were used to analyze differences in prey capture rate between cut and uncut traps.

Results:

For the lab tests, it was found that traps less than 1.0 cm in length did not respond well to surgery and typically did not reopen or turned brown and shriveled up. For the field experiments traps that were larger than 1.0 cm in length were used in order to minimize the chance of death by the trap.

Each individual plant was given a number. Experiment one had individuals EM1-EM11 while experiment two had individuals EM20-EM39. Some traps caught several prey items

Experiment one was analyzed with and without EM8 to see if there was a significant difference in data. The paired t-test with EM8 showed that there was not a significant difference in trap length or prey length in cut and uncut traps ($p=0.63$, $p=0.21$). Removing EM8 from the data and conducting a paired t-test yielded the same result, there was not a significant difference in trap or prey length between cut and uncut traps ($p=0.72$, $p=0.27$). Figure 1 shows the average prey length of captured prey in both cut and uncut traps. The differences in the averages were

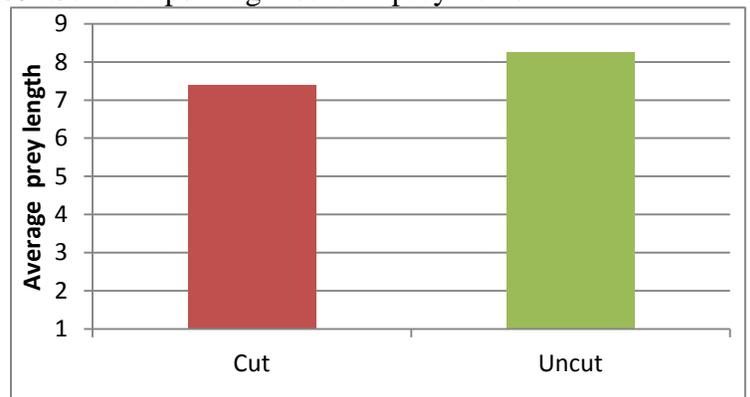


Figure 1: Average prey length of cut and uncut traps. Cut traps (red) average=7.38, using average prey length of prey items caught in EM8. Uncut traps (green), average=8.25.

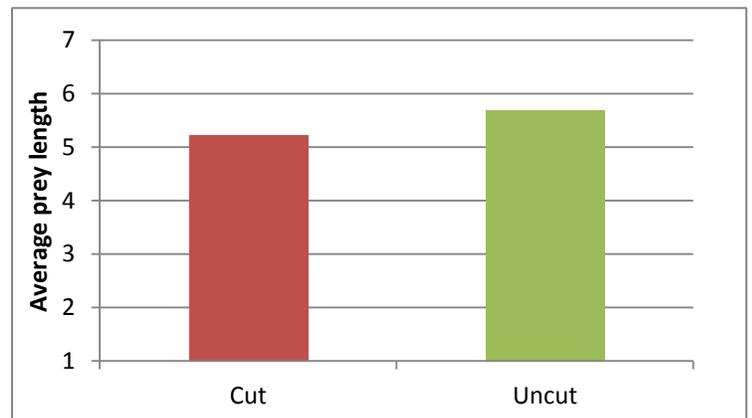


Figure 2: Average prey length of cut and uncut traps. Cut traps (red) average=5.2. Uncut traps (green) average=5.6

not statistically significant ($p=0.425$)

In experiment two, differences in trap length and prey length captured by cut and uncut traps was not statistically significant ($p=0.35$, $p=0.48$). Figure 2 shows the average prey length of captured prey items between cut and uncut traps, there difference was not statistically significant ($p=0.254$).

The most common prey caught for cut traps in both experiment one and two was Formicidae (56). All 56 ants were caught by EM8. Without EM8, the most common prey captured by cut plants was a tie between Araneae, Hemiptera, and Coleoptera (5), while Araneae (8) was the most commonly caught prey for uncut traps in both experiment one and experiment two.

Tables one and two show the number of traps that closed throughout the duration of the experiment compared to how many traps caught prey. The difference in prey capture rate between cut and uncut traps was not significant in experiment one nor experiment two ($X^2=0.1323$, $p>0.05$, $X^2=0.500$, $p>0.05$).

Table 1: Number of traps that closed in each treatment compared to how many caught prey in Experiment 1. Numbers in parenthesis are expected values for the Chi-square test.

| Experiment #1 | Number of traps closed | Number of traps that caught prey |
|---------------|------------------------|----------------------------------|
| Cut | 9 (9.55) | 6 (5.45) |
| Uncut | 19 (18.45) | 10 (10.5) |
| Total | 28 | 16 |

Table 2: Number of traps that closed in each treatment compared to how many caught prey in Experiment 2. Numbers in parenthesis are expected values for the Chi-square test.

| Experiment #2 | Number of traps closed | Number of traps that caught prey |
|---------------|------------------------|----------------------------------|
| Cut | 20 (20.48) | 13 (12.5) |
| Uncut | 34 (33.5) | 20 (20.48) |
| Total | 54 | 33 |

Discussion:

This experiment led to a few unexpected outcomes. One trap on EM8 caught 56 prey items. Each was a member of Formicidae, and usually less than 2mm in length. Since this was a rare phenomenon, data analysis was conducted with and without this specimen to get a better understanding of prey capture in Venus flytraps. Paired t-tests with and without EM8 yielded the same result, there was not a significant difference between trap length and prey length between cut and uncut traps (trap length: $p=0.63$, $p=0.72$, prey length $p=0.21$, $p=0.27$). This shows that prey lengths were not different in cut and uncut traps. If the cilia influenced the length of prey that was caught, the cut traps should have captured larger prey than the uncut traps because the cilia were not there to keep smaller prey in. Including the prey captured from EM8, the average size of prey captured by the cut traps was 3.2mm while the uncut was 8.25. Using the average size of prey captured by EM8, the average size did increase to 7.38, but this was not a significant difference. For experiment one, the cilia did not influence the size of prey captured by Venus flytraps. After performing a t-test on the data from experiment two, it was evident that there was not a significant difference in trap length or prey length between the cut and uncut traps ($p=0.35$, $p=0.48$). These data supports the hypothesis that the cilia do not have a significant impact on the size of prey captured by the Venus flytrap.

Another trap located on the same individual (EM8) captured a 10 mm salamander. According to some literature, carnivorous plants occasionally capture small amphibians and reptiles (Rice, 2002). It is strange that both phenomena occurred on the same individual. The two were collected on different days so they are probably not related. Perhaps there could have been some sort of attractant near that particular Venus flytrap which led to high organism traffic.

The cilia also did not affect prey capture rates. Since it is widely accepted that the cilia prevent large prey from escaping, it must have an overall better prey capture rate because the cilia block the exit, forcing prey to remain inside. According to the Chi square tests, there was no difference in the number of traps that closed and captured prey in cut and uncut treatments in either experiment one or experiment two. The expected values are almost identical to the observed values, which supports my other hypothesis, that cilia do not affect prey capture rates in the Venus flytrap. The uncut traps closed more than the cut traps did, this could mean that the experimental methods may have had adverse effects on the overall trap, causing the traps to become less sensitive to environmental stimuli.

Previous research has stated that the cilia automatically select for prey of certain sizes (Gibson, 1991). This experiment supports the hypothesis that cilia do not have an effect on the size of prey captured by traps with and without cilia. The trigger hairs would most likely be the evolved trait of the Venus flytrap that selects the prey item based on its size. Small insects are much lighter than large ones, so a light insect may not exert enough pressure onto the trigger hair to cause an action potential. If this small insect was able to press a trigger hair, the whole leaf is desensitized for three quarters of a second. If during that time a second trigger hair is pressed, the trap will not close. If a trigger hair is pressed after that time, then the trap will snap shut (Brown and Sharp, 1910). The very brief period of time that the trap is desensitized to stimulus of the

trigger hairs seems to be a built-in filter. If an insect is able to press two hairs within three quarters of a second, then it may be too quick for the trap to consume. If it is able to get to trigger hairs that are a good distance apart, from a small bug's perspective, it could probably escape the whole trap within that time frame.

Since small insects are unlikely to be able to create enough force to press down the trigger hairs the trap will not close for insects of that size to begin with, then the cilia would not be an evolved trait to keep insects inside the trap. Closing the trap for a small insect would lead to missed feeding opportunities for the plant. Traps are only able to close a few times over their lifespan, so closing the trap to receive zero nutrients would be detrimental to the survival of the plant.

Venus flytraps have evolved to have a very quick closing speed. The closing speed is about 0.3 seconds (Volkov et al., 2011). This seems to be a better mechanism to ensure prey cannot escape. The distance that needs to be traveled by the escaping insect is small, but since the trap closes so quickly it would still be too far to travel to escape. The rapid closing speed does not seem to give organisms enough time to register that anything is happening.

Further experiments need to be conducted in order to understand the role of cilia in trapping for Venus flytraps. I would not suggest conducting an experiment similar to experiment 1 because it was found that new traps grew in during the span of the experiment which could have resulted in the uncut group appearing to have a better prey capture rate than the cut group, although according to Tables 1 and 2 there was no difference. If this experiment was redone, I would suggest going back to the site and monitoring the growth of new traps more carefully. If the traps were looked at every two or three days then new traps could be removed at the first

sighting. Another problem with experiment one was that the sample size was rather small because on some plants only a cut trap caught prey while on others neither cut nor uncut traps caught prey. For the analysis those plants were left out, resulting in eight observations for trap lengths and four for prey lengths. The sample size of experiment two was larger, making the results more credible. Future studies could be conducted in a manner similar to experiment two, but with a larger sample size, over a longer time, during a different season and with larger and smaller traps. There could be other sized insects available during other times of the year that require the cilia in trapping. As for the summer, it does not seem evident that the cilia play a significant role in prey capture.

Literature cited

- Adamec, L. 2002. Leaf absorption of mineral nutrients in carnivorous plants stimulates root nutrient uptake. *New Phytologist* 155:89–100
- Affolter, J.M., and R.F. Olivo. 1975. Action potentials in the Venus's-flytraps: Long term observations following the capture of prey. *American Midland Naturalist* 9:443-445.
- Albert, V.A., S.E. Williams, and M.W. Chase. 1992. Carnivorous plants: Phylogeny and structural evolution. *Science* 257:1491-1495.
- Brown, W.H., and L.W Sharp. 1910. The closing response in *Dionaea*. *Botanical Gazette* 49:290-302
- Darwin, C. 1888. *Insectivorous Plants*. 2nd edition. William Clowes and Sons. England.
- Ellison A.M. 2006. Nutrient limitation and stoichiometry of carnivorous plants. *Plant Biology* 8:740-747
- Ellison, A.M., and N.J Gotelli. 2001. Evolutionary ecology of carnivorous plants. *Trends in Ecology and Evolution* 11:623-629.
- Fagerberg, W.R., and D.G. Howe. 1996. A Quantitative study of tissue dynamics in Venus flytrap *Dionaea muscipula* (Droseraceae). II. Trap reopening. *American Journal of Botany* 83:836-842.
- Forterre, Y., J.M. Skotheim, J. Dumais and L. Mahadevan. 2005. How the Venus flytrap snaps. *Nature* 433:421-425.
- Gibson, T.C. 1991. Differential escape of insects from carnivorous plant traps. *American*

- Midland Naturalist 125:55-62.
- Givnish, T.J., E.L. Burkhardt, R.E. Happel and J.D. Weintraub. 1984. Carnivory in the bromeliad *Brocchinia reducta*, with a cost/benefit model for the general restriction of carnivorous plants to sunny, moist, nutrient-poor habitats. *The American Naturalist* 124:479-497.
- Hutchens, J.J., and J.O. Luken. 2009. Prey capture in the Venus flytrap: Collection or selection? *Botany* 87:1007-1010.
- Jaffe, M.J. 1973. The role of ATP in mechanically stimulated rapid closure of the Venus's-flytrap. *Plant Physiology* 51:17-18.
- Jeschke, J.M., M. Kopp, and R. Tollrian. 2004. Consumer-food systems: why type I functional responses are exclusive to filter feeders. *Biology Revolution* 79:337-339.
- Jürgens, A., A.M. El-Sayed and D.M. Suckling. 2009. Do carnivorous plants use volatiles for attracting prey insects? *Functional Ecology* 23:875-887.
- Lichtner, F.T., and S.E. Williams. 1977. Prey capture and factors controlling trap narrowing in *Dionaea* (Droseraceae). *American Journal of Botany* 64:881-886.
- Luken, J.O. 2007. Performance of *Dionaea muscipula* as influenced by developing vegetation. *The Journal of the Torrey Botanical Society* 134:45-52.
- Rice, Barry. 2002. Carnivorous plants- classic perspectives and new research. *Biologist*. 49:1-5.
- Roberts, P.R. and H.J. Oosting, 1958. Responses of Venus flytrap (*Dionaea muscipula*) to factors involved in its endemism. *Ecological Monographs* 28:193-218.
- Scala, J., K. Iott, D.W. Schwab, and F.E. Semersky. 1969. Digestive secretion of *Dionaea muscipula* (Venus flytrap). *Plant Physiology* 44:367-371.

Volkov, A.G., M.R. Pinnock, D.C. Lowe, M.S. Gay, and V.S. Markin. 2011. Complete hunting cycle of *Dionaea muscipula*: Consecutive steps and their electrical properties. *Journal of Plant Physiology* 168:109-120