Investment in Carnivory in *Drosera capillaris*:
Effects of Varying Light Levels on Trichome Density

by

Rebecca Lee Godwin

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Approved by

Debbie R. Folkerts, Chair, Assistant Professor of Biology
Robert S. Boyd, Professor of Biology
Robert S. Lishak, Associate Professor of Biology
Sharon M. Hermann, Visiting Assistant Professor of Biology

Abstract

In this study I examined *Drosera capillaris*, which is a plant common in the southeastern Gulf Coastal Plain's pitcher plant bogs. Investment in carnivory has been examined in other groups of carnivorous plants but very little investigation has been done in members of the genus *Drosera*. I considered trichome density as a potential indicator of investment in carnivory in *Drosera capillaris*. Since it can be assumed that reducing light reduces photosynthesis and subsequently can reduce the benefits gained from carnivory, I observed the effects of varying light levels on these plants. Two non-field experiments were conducted. In one, plants were placed in a greenhouse to determine the effects of varying light levels on trichome number and trichome density in a controlled setting. Results for this experiment were not conclusive, most likely due to a combination of small sample size and high plant mortality. In a second non-field experiment conducted in the lab, I examined rates of prey escape in *Drosera capillaris* to determine if reducing trichome density affected the plants' ability to retain prey. Prey escape trials using *Drosophila sp.* did not show a statistically significant trend toward higher prey escape rates for plants with reduced numbers of trichomes. The lack of significant results were most likely due to small sample size as well as the fact that *Drosophila sp.* are larger than prey normally captured by *Drosera capillaris*. In the field, I clipped surrounding vegetation and created plots in which light levels were greater to determine the effects of light level on trichome number and trichome density. In 2009 in TNC and Big Bog and in Lottie Bog in 2010, I found that increasing the amount of available light did not lead to a significant increase in trichome

density. However, data collected from Lottie Bog in 2009 showed a significant increase in trichome density in plants which had more available light. By changing the amount of light available to some plants within a population, I have been able to demonstrate that *Drosera capillaris* can respond to changes in the environment within a given season and population through alteration of investment in carnivory as measured by trichome density.

Acknowledgments

The author would like to thank her committee for all the guidance and advice they provided. Many students at Auburn University helped with field and lab work and the author would like to acknowledge Amy Grilliot, Jess Stephens, Nathan Hall, Andrew Smith, Erin Bishop Bilbo, Rob Smola, Kristen Peterman, Ansley Pike, Cole Nelson, Tim Littman, Jessica McMeekin, and Cassandra Jorgensen. The author would also like to acknowledge Leo Tsuji for his advice in the rearing of sundews and other carnivorous plants. The author is thankful to The Nature Conservancy, specifically Brian Martin and Keith Tassin, for their help and for the use of the house on the TNC property in Baldwin County. The author is also thankful to the Department of Conservation of Natural Resources for the use of one of their Forever Wild tract and to Dr. Kolb for the use of his property in Baldwin County. Finally, the author would like to thank her family, friends and husband for all their love and support.

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Introduction

Wet pine savannahs and seepage savannahs, commonly called pitcher plant bogs since these are the most apparent of the many carnivorous plants which inhabit them, are located across the Lower Gulf Coastal Plain (Folkerts 1982). Folkerts (1982) notes that these habitats are characterized by sandy, loamy, and generally strongly acidic soils that are saturated with water for at least a portion of the year. These are wetlands in which water movement is very slow, with water percolating out of hills and slopes from surrounding woodlands, either into depressed areas or out onto the sides of hills, where it then seeps down through the porous soil which is underlain by a clay hard pan or other impervious soil layer (Folkerts 1982).

Wet pine and seepage savannahs are found embedded within the forest of longleaf pines that historically covered approximately 90 million acres of the southeastern United States (Kush et al. 2004). Much of the longleaf forest has been lost due to the combined effects of development, logging and fire exclusion, and the carnivorous plant habitats within the ecosystem have fared no better, with conservative estimates stating that as much as 97% of the historic pitcher plant bog habitat has been completely lost or drastically altered (Folkerts 1982).

In the nutrient poor soils of the wetlands in which most carnivorous plants occur, carnivory provides a way for plants to supplement nutrients that they cannot get in sufficient amounts from the soil (Givnish 1989). Since carnivory does not replace, but merely facilitates photosynthesis, carnivory is expected to be adaptive only when photosynthesis is not limited by other factors such as light or water (Givnish et al. 1984). This explains why carnivorous plants exist primarily in wet and sunny habitats. It also explains why carnivorous plants do not thrive

in damaged habitats where light and moisture levels have been altered due either to fire exclusion or development.

Carnivorous plants rapidly reappear after fire. *Drosera* species may be some of the first plants to begin to die out when the bog becomes overgrown from poor management or insufficient burning or when a bog becomes dry either from being drained or from drought. Currently, carnivorous plants in the southeastern United States are threatened by fire exclusion, agriculture and forestry, over-collecting, and climate change (Ellison and Gotelli 2001). Research is needed to better understand these plants and to guide conservation and management.

In this study, I examined the pink sundew, *Drosera capillaris*, a carnivorous plant that produces what is commonly referred to as "flypaper traps" (Givnish 1989) *Drosera* spp. are characterized by a basal rosette of normally recumbent leaves, each of which bears a number of stalked, multicellurlar trichomes that produce a viscous mucilage and are used in prey capture as well as shorter digestive glands (Darwin 1875, Pietropaolo and Pietropaolo 1986, Schnell 2002). While many *Drosera* spp. are easily shaded-out and cannot compete with encroaching plant species when bogs become overgrown from lack of fire or moisture, they are also some of the first plants to reappear after an area has been opened up, whether by cutting or burning (Schnell 2002).

One area of interest is whether investment in carnivory varies under varying environmental conditions. As stated above, carnivory is only advantageous when other factors, such as light or water, are not limiting photosynthesis. I accept the assumption that the carnivorous habit is costly so I predicted that investment in carnivory would be lowered when other factors limit photosynthesis.

Studies on investment in carnivory must be approached carefully. This is due to the fact that with most carnivorous plants (such as venus fly traps, pitcher plants, butterworts and sundews), the trapping leaves are also photosynthetic. This potentially confounds investment in carnivory with investment in photosynthesis and requires that parameters of measurement of investment be chosen carefully.

Work has been conducted in *Utricularia* species, or bladderworts. The bladderworts lend themselves to investigations of investment in carnivory because their trapping mechanisms, the bladders, are separate from their photosynthetic portions. Bladder size and number and antennae length vary in response to alterations of factors such as light and nutrient levels (Englund and Harms 2003, Friday 1992, Manjarres-Hernandez et al. 2006). Englund and Harms (2003) found that *Utricularia* grown in conditions of low light and high nutrients invested very little in carnivory and produced no bladders. *Utricularia* grown in low nutrient conditions showed a high investment in carnivory (Guisande et al. 2004). Studies have documented a change in investment in carnivory in *Utricularia* over the course of a growing season. Friday (1992) and Knight and Frost (1991) found that *Utricularia* is able to acclimate during a growing season and adjust its investment in carnivory. However, Manjarres-Hernandez et al. (2006) found that difference in investment in carnivory depends more on the environmental location in which the plants occur than on changes during a growing season.

Studies on investment have been conducted on other groups of carnivorous plants as well. In a study of *Sarracenia purpurea* (Ellison and Gotelli 2002) increased nitrogen availability led to a reduction in pitcher production. *Pinguicula*, or butterwort, another "flypaper trap" type plant, exhibits trichome density as a plastic character which varies under differing environmental conditions (Zamora et al. 1998, Alcalá and Domínguez 2003, 2005) and so the feature of

trichome density can be used as a potential measure of investment in carnivory. Zamora et al. (1998) conducted a study examining effects of prey and light availability on investment in carnivory in *Pinguicula* as measured by density of glands and size of mucilage droplets. They found that the density of glands was only affected by, and positively correlated with, abundance of prey. Increases in both light and abundance of prey led to an increase in the size of mucilage droplets (Zamora et al. 1998). Alcalá and Domínguez (2005) conducted a study of density of trapping and digestive trichomes in multiple populations of *Pinguicula* across an environmental gradient. They found that *Pinguicula* in areas with lower amounts of available nutrients and higher levels of available light produced the greatest densities of both types of trichomes.

In a study of the roundleaf sundew, *Drosera rotundifolia*, light and nutrient levels appeared to influence investment in carnivory (Thoren et al. 2003). The authors chose stickiness of the leaves as their parameter of investment in carnivory. They found that decreasing light decreased investment in carnivory, which coincides with expectations from the Givnish et al. (1984) model. However, Schnell (2002) suggested mucilage production is greatly affected by humidity and moisture, and Darwin (1875) observed that mucilage was produced in response to the presence of potential prey items, and that more and different mucilage was produced depending on objects placed on the leaves. Thoren et al. (2003) admit that their measurement of stickiness was "rough," at best. Stickiness, as they measured it, is likely a function of mucilage viscosity as well as trichome density. I feel that measurement of trichome density more clearly indicates investment in carnivory in *Drosera* spp. and is not confounded by other factors.

In this study, I chose to examine *Drosera capillaris*, the pink sundew, a small herbaceous forb common in the seepage and wet pine savannas of the southern gulf coastal plain. Trapping structures of *Drosera capillaris* are components of their photosynthetic leaves but are separate in

function. Trichomes play a role in trapping prey in this genus. Trichome density is a quantifiable character, and I believe that it is therefore a potential indicator of carnivory investment.

To date, studies in fly-paper type traps (i.e. *Pinguicula* spp. and *Drosera* spp.) have concentrated on variation between populations with regard to trichome density (Acalá and Domínguez 2005, Thoren et al. 2003). It has not been shown whether or not the variation in trichome density or investment in carnivory is a set genetic trait, specific to each population or if it is a phenotypically plastic trait which would allow plants within a population to acclimate to changing environmental conditions. Using the density of this leaf feature as a measure of investment in carnivory, I designed a number of experiments to attempt to answer this question. I conducted an experiment in a greenhouse, where a shading treatment was applied to assess the influence of varying light levels on the density of trichomes in a relatively controlled environment. To better understand whether or not trichome density is an effective measure of investment in carnivory, I designed a prey escape experiment in which trichome density in treatment plants was manipulated to test whether reduction in trichome density resulted in a reduction of the prey retention ability of leaves. In the field, to assure that my treatments were not resulting in etiolation or general decreased health of the study plants, I tracked changes in adult size, as measured by maximum rosette diameter and number of leaves per plant of a total of 800 plants over portions of two field seasons. I assessed the influence of light on the density of trichomes in the field by removing surrounding shading vegetation in treatment plots. I clipped all plants, except *D. capillaris*, to partially mimic the increased light at ground level following a fire.

I am interested in both theoretical and practical aspects of carnivorous plant biology and wetland preservation. I intend to study the influence of light, a factor related to both investment

in carnivory and habitat management. By increasing available light to plants by removing shading vegetation I can partially mimic the effects of fire (Brewer 1998). In choosing this variable I am able to answer questions on variable investment in carnivory and also to enhance our understanding about these plants' response to habitat conditions and subsequently our preservation and management techniques.

I predicted that shaded and non-shaded plants would have different trichome densities by the end of each field season. I also predicted that mechanically decreasing trichome density on individual leaves would decrease a leaf's ability to retain prey. I predicted that trichome density is plastic within populations over time. When available light is increased, I expected investment in carnivory to increase since nutrients gained from carnivory would be needed as more photosynthesis is taking place due to the increased light. Results from these experiments were expected to provide insight as to what degree investment in carnivory varies under varying environmental conditions, specifically light.

Materials and Methods

Non-Field Experiments

Greenhouse Trichome Experiment – To examine the effects of differing light levels in a relatively controlled setting, plants were subjected to manipulated shading in a greenhouse from October to December of 2010. Plants were collected from a roadside population in Baldwin County, Alabama, held temporarily in a growth chamber and later placed in a greenhouse. Five plants were transplanted into each of four 8" round pots. The pots were then placed in plastic trays with 1-2 inches of water. Shade cloth tents were placed over all of the pots for one week to help them acclimate to greenhouse conditions. After one week, shade cloth was removed from two of the pots, exposing plants to full greenhouse sun, creating the unshaded treatment. A shade tent with 50% shade cloth was left on the remaining two pots. In each of the pots, marked 1-4, rosette diameter and number of leaves were recorded for all plants, marked A-E in each pot. Leaf samples were taken from the five plants by removing an entire leaf and petiole with micro-forceps. Growth measurements and leaf samples were taken once every two weeks. Leaf samples were collected by removing leaves at the base of the petiole so as not to remove or disturb trichomes on the main body of the leaf. Samples were stored in ethanol until they could be processed in the lab. Trichome counts were done by removing and counting trichomes under a dissection microscope. Using the number of trichomes counted and the area of the leaf, which was calculated by treating the leaf as a circle and measuring the diameter of each leaf, density of trichomes per mm^2 were recorded for all leaf samples.

A One-Way ANOVA (Stat View 5.0.1) was used to determine if treatment had a significant effect on trichome densities in the first and last days of treatment.

Prey Escape Experiment – In 2010 I conducted a laboratory experiment to examine the effect, if any, of trichome density on prey escape. For this experiment, plants were collected from a roadside in Baldwin County, Alabama. Plants were transplanted into 3" deep jars so that the soil surface was nearly level with the top of the jar. The jars were then placed in a growth chamber.

The experiment was done in seven runs of two pairs, each pair consisting of one treatment and one control plant for a total of 14 treatment and 14 control plants. Pairs were chosen so that both plants had an equal number of leaves. Twenty trichomes were removed from each leaf of treatment plants, using micro-forceps, to reduce trichome density. I conducted trial runs to determine how many trichomes could be removed from leaves without causing immediate damage to the leaves. Trichomes were removed evenly from across all areas of the leaf so that density would be reduced as evenly across the entire surface of the leaf as possible. The pairs of plants were then placed in mosquito chambers in the growth chamber and left overnight so that the manipulated plant had time to produce mucilage. On the second day fruit flies were anesthetized with ether. One fly was placed feet down on the center of every leaf. The plants were returned to the mosquito chambers and placed back in the growth chamber for 24 hours. After 24 hours, plants were removed from the mosquito chambers and the number of retained prey (flies which did not escape the leaf) per leaf/per plant were recorded.

I used a Chi-Square test (SAS 9.1) considering the total number of escaped flies and compared those which escaped from control leaves versus those which escaped from treatment leaves.

Field Experiments

Field Sites – Experiments were conducted in 2009 and 2010. Three bogs were used in 2009, Big Bog, TNC Bog and Lottie Bog. All three bogs are located in the Splinter Hill Bog System in Baldwin County, Alabama. Due to prescribed burns in two of the bogs, only Lottie Bog was used in 2010.

Big Bog is a Forever Wild Tract managed by the Alabama Department of Conservation and Natural Resources. This large bog is relatively open and rolling in topography with few *Pinus palustris*. *Smilax* was relatively abundant along the edges in this bog. Big Bog has been burned every two years for the last 14 years. A growing season burn was conducted in the summer of 2009, but the area of the bog containing my plots was avoided. Some smaller burns and maintenance were also being done in the spring of 2010 causing this bog to not be used for my second field season.

A second bog which I will refer to as TNC Bog, was used only during the first field season (2009) and is owned by The Nature Conservancy (TNC). This bog is located within a wooded area in the Nature Conservancy property with a relatively thick overstory of *Pinus* spp. This bog was much more overgrown with shrub species such as *Ilex* and also with *Smilax*. The diversity of bog plants is higher in this bog than in Lottie Bog. The bog is also located on a slight slope. TNC conducted a growing season burn in late May 2010 so I was not able to use this site for my second field season.

Lottie Bog is a privately owned bog and was used in both years. The bog had not been burned in at least the past four years prior to the study. Shrub species (e.g. *Ilex* spp.) were beginning to encroach around the edges of the bog. There was a lot of built up fuel (matted

grass, old pitchers, etc) on the ground. The bog is relatively flat and open, with few trees (*Pinus palustris*) that become more dense at the edge of the bog. The bog is bordered at one side by a paved road.

Field Site Set-Up – In May of 2009 I created plots in Lottie Bog, TNC Bog and Big Bog. In each bog ten pairs of $1m^2$ plots were marked with pin flags and flagging tape. To determine placement of plots, I located patches where *Drosera capillaris* was sufficiently dense to accommodate ten marked plants with at least ten extra plants to collect leaf samples. There was approximately 0.5m between plots within a pair and each pair was at least 1m from an adjacent pair of plots. I marked ten plants in each of the twenty plots in each of the three bogs with aluminum numbered tags. I then used garden shears to clip all of the herbaceous growth as close to the soil surface as possible to create my treatment or "clipped plot" in one of each of the ten pairs. The other plot of each pair was left as a control. Clipped plots were re-clipped on each data collection visit to each bog to maintain the reduced shade of the clipped plots.

In April of 2010 I created plots only in Lottie Bog for my second field season. Ten pairs of plots were created and maintained in the same manner as the first season in a different section of the bog, but at approximately the same distance (25m) from the edge of the bog.

Effect of Treatment on Light - To assure that clipping herbaceous growth created a significant difference in light levels between clipped and unclipped plots; relative measurements of available light were recorded for all three bogs once in August of 2010 using a LiCor LI 250 light meter with a Quantum sensor. The sensor was uncalibrated and so was used to record unitless light levels which were only analyzed relative to each other. Light levels in Big Bog and

TNC Bog were recorded in each bog at ground level beneath shading herbaceous growth and at breast height along a straight line through the center of the bog at two meter intervals. In Lottie Bog measurements were made in each clipped and unclipped plot at ground level and at breast height. All measurements were taken three times over the course of the day: in the morning around 8:00am, at noon and in the early evening around 4:00pm.

Two-Way ANOVAs (PROC GLM, SAS 9.1) were used to compare light levels in each bog at breast height and ground level and between breast height and ground level in clipped plots in Lottie Bog. Light levels were analyzed as the dependent variable with location of measurement and time of day as factors.

Effect of Clipped vs. Unclipped Treatments on Changes in Adult Plant Size – In order to help determine that differences in trichome densities between clipped and unclipped plots were not due to etiolation effects or change in plant health due to excessive or insufficient light, I measured diameter and number of leaves for each of the tagged and numbered plants over the course of my field season. Measurements were taken in the 2009 season on May 17, May 30, June 14, August 1, and August 30 in all three bogs, exceptions being on May 17 and August 30 when weather did not permit data collection in Big Bog as it was inaccessible.

I used One-Way ANOVAs (PROC GLM, SAS 9.1) to determine if rosette diameter or number of leaves were different over time in clipped versus unclipped plots. In all bogs, clipped and control plots were treated separately with respect to trichome density.

Effects of Clipped vs. Unclipped Treatments on Trichome Density - Leaf samples were taken on May 2, 17 and 30, June 14 and 28, and August 1 and 30 during the 2009 field season and were taken monthly from May through and including August during the 2010 field season. Five entire leaves, including petiole, were removed with micro-forceps from each of five adult unmarked plants in each plot. Samples were brought back to the lab in 70% ethanol and stored in a refrigerator. Trichome density was determined for each leaf by removing and counting all of the trichomes on the leaf under a dissecting microscope. Leaves had varying numbers of trichomes on their petioles, which were not included in these calculations, as they were not located on the main leaf body. Upper surface leaf area was calculated using the maximum diameter and assuming that the leaves were roughly circular. Number of trichomes and leaf area were used to calculate trichome density.

Two-Way ANOVAs (PROC GLM, SAS 9.1) were performed to determine if treatment of plots had a significant effect on trichome densities in the first and last days of treatment. Trichome density was analyzed as the dependent variable with treatment (clipped vs. unclipped) and plot included to account for a possible block affect. Tukey's test (SAS 9.1) was used to determine groupings of trichome densities among dates.

Results

Non-Field Experiments

Greenhouse Trichome Experiment - One-Way ANOVAs were used to compare trichome density in shaded and unshaded plants on the initial and final days of treatment. In the initial day of treatment there was no significant difference between shaded and unshaded plants ($df=1$, $p=0.1056$). On the final day of treatment there was also no significant difference between shaded and unshaded plants ($df=1$, $p=0.4867$). Greenhouse sample size was small (only one plant survived until the final day of sampling in the unshaded plants) and most plants perished before the end of the experiment.

Prey Escape Experiment – The total number of escaped flies for both treatments was 141 (62 escaped in control leaves and 79 in treatment leaves). Comparing the number of escapes from treatment versus control leaves gave a Chi-Square value of 2.0496 ($p=0.1552$). Percent of flies escaped for each trial are presented in Figure 1.

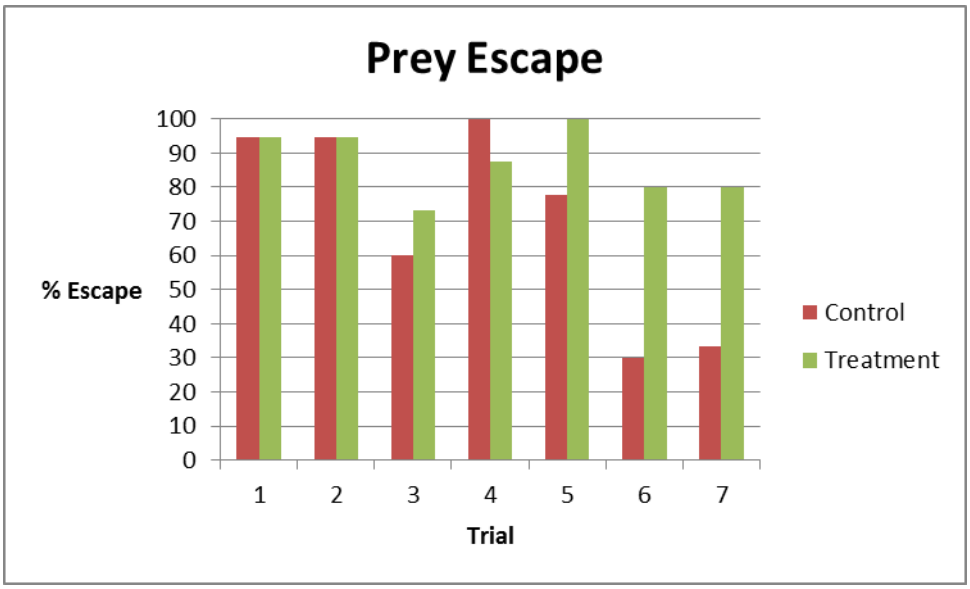


Figure 1. Percent of flies escaped for each trial. Two control and two treatment plants were used in each trial. A total of 186 flies were placed on individual leaves of plants, with 93 flies for each treatment.

Field Experiments

Effect of Treatment on Available Light – Light levels were recorded for each of the three bogs at roughly 8:00am, noon and 4:00pm, and the recorded data was analyzed using Two-Way ANOVAs to determine the effect of condition (breast height, ground level in all bogs and ground level in clipped plots in Lottie Bog) on light level with time included as a second factor. The raw data from the sensor is presented in Figure 2. Light level at breast height was lower in TNC Bog than in Big Bog or Lottie Bog. Light levels were significantly different between breast height and ground level in all three bogs (Fig. 3a-c); Big Bog ($F=188$, $p<0.0001$), Lottie Bog ($F=52.8$, $p<.0001$) and TNC Bog ($F=42.2$, $p<0.0001$). In Lottie Bog light levels recorded at ground level in clipped plots (with shading herbaceous vegetation removed) were not significantly different from light levels at breast height (Fig. 3c).

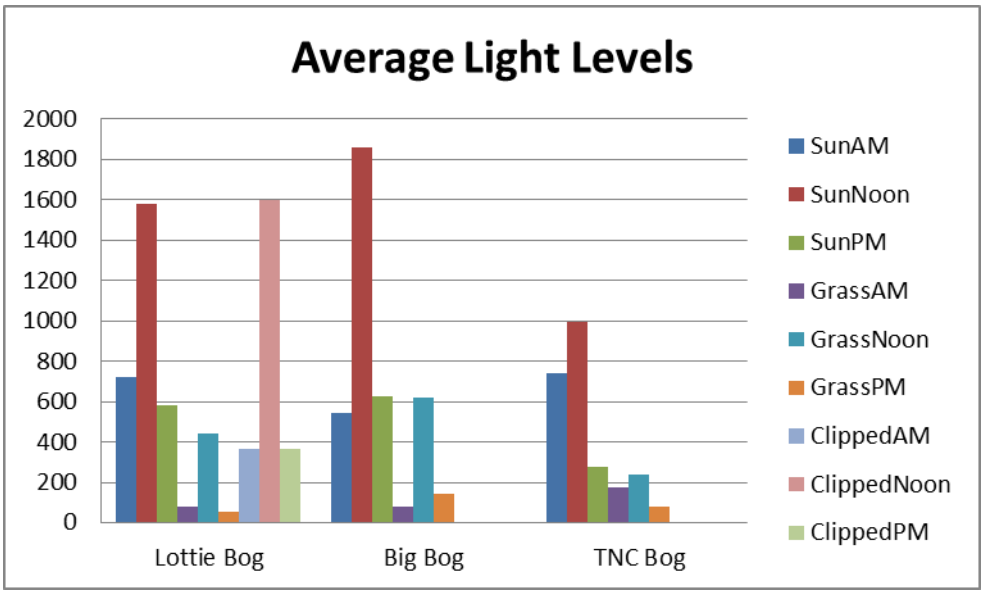
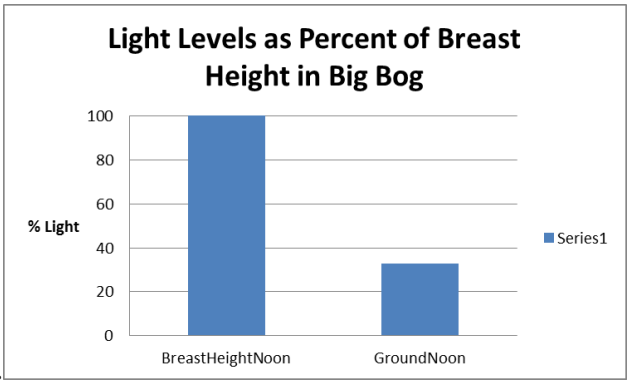


Figure 2. Unitless light levels for three times of day at breast height (Sun) and ground level (Grass) for all three field sites and at ground level in clipped plots (Clipped) in Lottie Bog

a.



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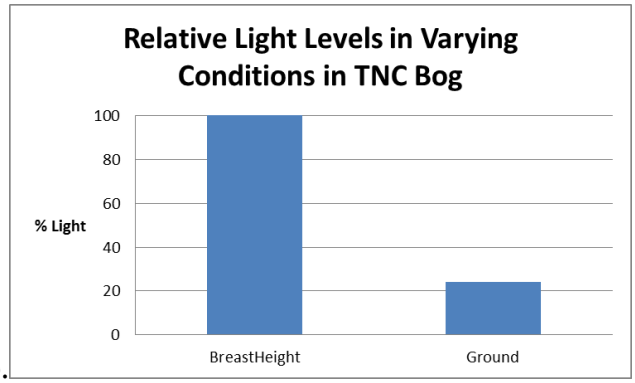

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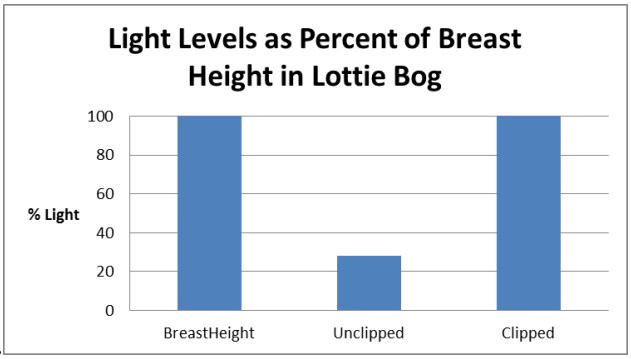


Figure 3a-c. Light levels as a percent of Breast Height for each bog.

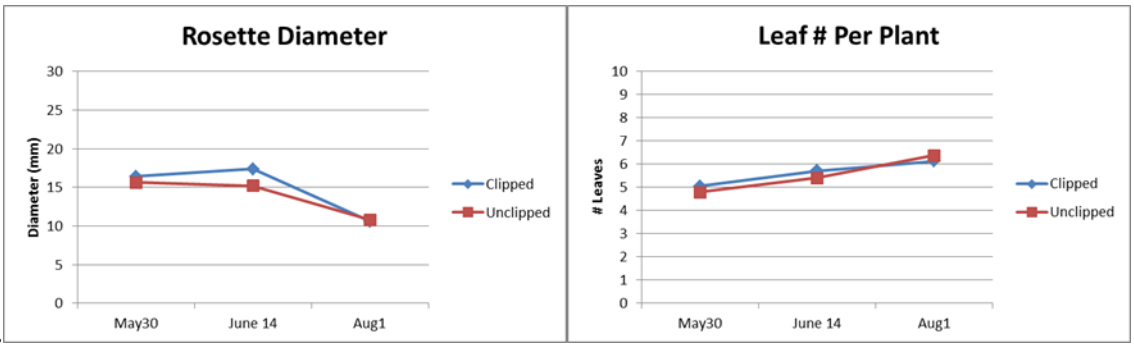
Effect of Clipped vs. Unclipped Treatments on Adult Plant Size - In Big Bog in 2009 maximum rosette diameter differed significantly when all factors are considered (df=5, F=33.8, p< 0.0001). Treatment (whether clipped or unclipped plots) had a significant effect on this (df=1, F=7.3, p=0.0070), and date for measurement of rosette diameter had a significant effect (df=2, F=77.7, p<0.0001). Number of leaves also differed significantly over the course of the field season in Big Bog (df=5, F=7.97, p= <0.0001), but treatment category had no significant effect on this change over the field season (df=1, F=0.76, p=0.3848). Date at which leaves were counted had a significant effect (df=2, F=18.7, p<0.0001). See Figure 4a.

In TNC Bog in 2009 maximum rosette diameter differed significantly when all factors are considered (df=9, F=2.01, p= 0.0355). Treatment category had no significant effect on this change (df=1, F=1.03, p=0.3097). Date for measurement of rosette diameter had a significant effect (df=4, F=3.96, p=0.0034). Number of leaves differed significantly over the course of the field season (df=9, F=20.8, p <0.0001). Treatment had no significant effect on this change (df=1, F=0.07, p=0.7853). Date at which leaves were counted had a significant effect (df=4, F=46.6, p<0.0001). See Figure 4b.

In Lottie Bog in 2009 maximum rosette diameter differed significantly when all factors are considered (df=9, F=42.3, p= <0.0001). Treatment had no significant effect on this change (df=1, F=2.2, p=0.1385). Date of rosette measurement had a significant effect (df=4, F=93.1, p=<0.0001). Number of leaves differed significantly over the course of the field season (df=9, F=13.7, p=<0.0001). Treatment had no significant effect on this change (df=1, F=1.6, p=0.2055). Date at which leaves were counted had a significant effect (df=4, F=27.3, p<0.0001). See Figure 4c.

In Lottie Bog in 2010 maximum rosette diameter differed significantly when all factors are considered (df=9, F=42.6, p<0.0001). Treatment category did have a significant effect on this change (df=1, F=0.16, p=0.0015). Date of measurement had a significant effect (df=4, F=89.8, p=<0.0001). Number of leaves differed significantly over the course of the field season (df=9, F=3.9, p= <0.0001). Treatment had no significant effect on this change (df=1, F=1.6, p=0.2124). Date on which the leaves were counted had a significant effect (df=4, F=6.02, p=<0.0001). See Figure 4d.

a.



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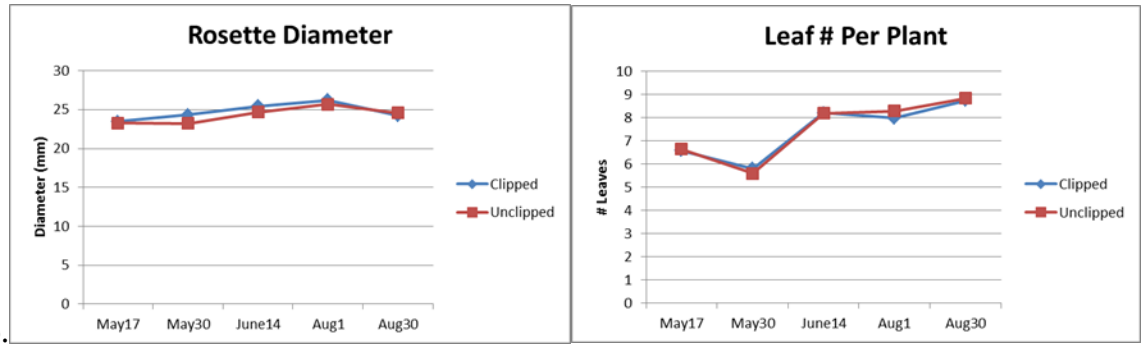

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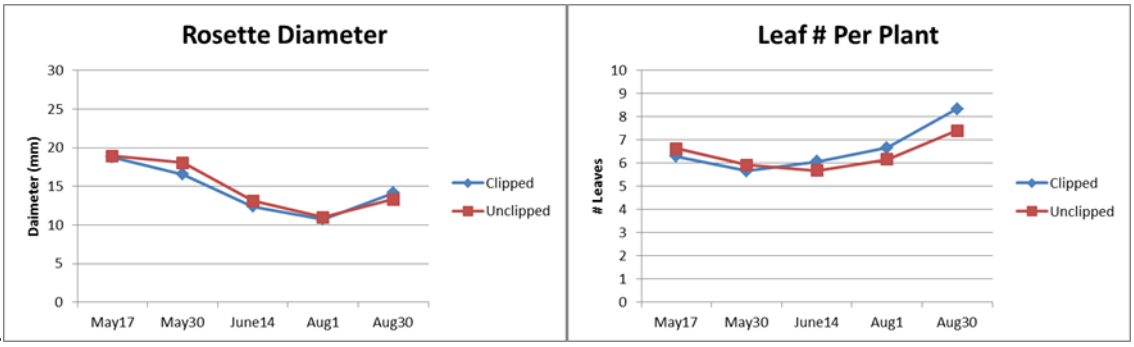


d.

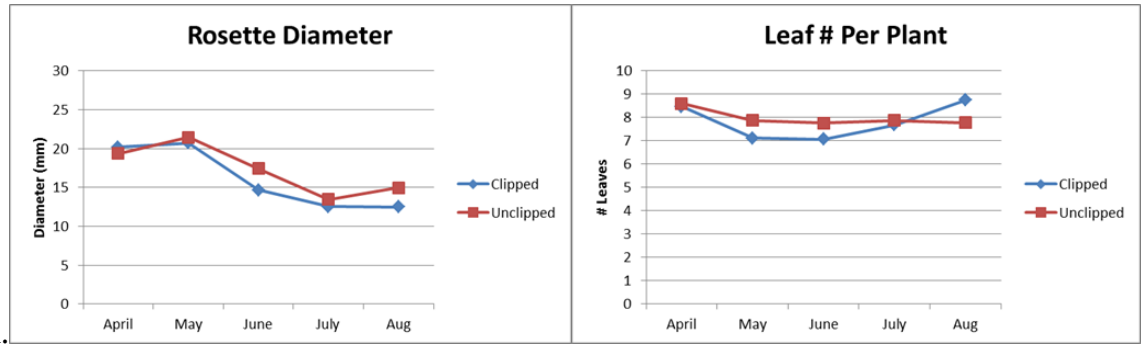


Figure 4a-d. Growth patterns in clipped and unclipped plots in a. Big Bog 2009, b. TNC Bog 2009, c. Lottie Bog 2009, and d. Lottie Bog 2010. Graphs show change in plant diameter and number of leaves over time in clipped and unclipped plots.

Effects of Clipped vs. Control Treatments on Trichome Density – Two-Way ANOVAs were used to analyze the effect of clipped versus unclipped treatments on trichome densities, including plots as a factor to account for any block effect. Both the initial and final days of treatment were analyzed seperately (Table 1).

Plot had no significant effect on trichome density in any of the three bogs on either the initial or final days of treatment except for in Lottie Bog (df=9, F=3.1, p=0.0031). There was no significant interaction between treatment and plot in any bog.

Trichome density in Big Bog in 2009 significantly differed between clipped and unclipped plots on the initial (df=1, F=16.6, p=0.0001) but not the final (df=1, F=0.02, 0.8981) days of treatment. In 2009 in TNC Bog trichome density was not significantly different between treatment and control plots on the initial day (df=1, F=2.4, p=0.1236) nor on the final day (df=1, F=1.05, p=0.3093) of treatment. In Lottie Bog in 2009 trichome density was not significantly different between clipped and unclipped plots on the initial day of treatment (df=1, F=1.04, p=0.3102), but was significantly different on the final day of treatment (df=1, F=17.58, $p<0.0001$). In 2010 in Lottie Bog trichome density was not significantly different between clipped and unclipped plots on the initial day of treatment (df=1, F=0.13, p=0.065) nor on the final day of treatment (df=1, F=5.5, p=0.640).

Comparison of Trichome Densities in Clipped and Unclipped Plots on Initial and Final Days of Treatment						
	Initial (Average # Trich/mm²)			Final (Average # Trich/mm²)		
	Clipped	Unclipped	P Value	Clipped	Unclipped	P Value
Big Bog 2009	9.9	13	0.0001	22.2	22.5	0.8981
TNC Bog 2009	7	7.5	0.1236	11.6	12.5	0.3093
Lottie Bog 2009	9.3	9	0.3102	17.4	14.1	<0.0001
Lottie Bog 2010	12.4	12.7	0.064	21.4	19.1	0.64

Table 1. Comparison of mean number of trichomes/mm² in clipped and unclipped plots on initial and final days of treatment with P values from Two-Way ANOVAs looking at effect of treatment and plot on trichome density.

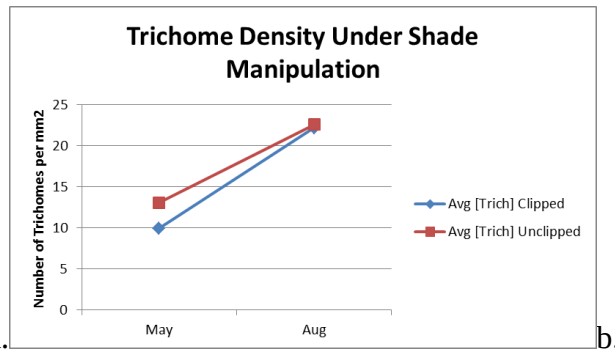
Patterns in Trichome Density Variation- Two-Way ANOVAs looking at the effects of condition and date on trichome density were used to analyze this data. Tukey's Studentized Range test was used to place dates into groups according to variation in average trichome densities.
In data collected for Big Bog in 2009 (Fig. 5a), clipped plots showed a significant increase in trichome density from May 2 to August 1 ($F=228.4$, $df=1$, $p<0.0001$). In unclipped plots there was also a significant increase in trichome density between data collected for May 2 and August 1 ($F=65.0$, $df=1$, $p=0.0001$) .

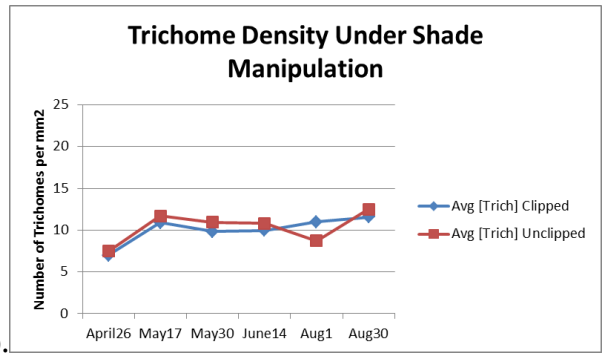
Data were collected in TNC Bog in 2009 (Fig. 5b) on April 26, May 17, May 30, June 14, August 1 and August 30. In clipped plots trichome densities were significantly different among dates ($F=21.2$, $df=5$, $p<0.0001$). Trichome density for April 26 was lower than all other dates, with trichome densities for May17, August 1 and August 30 falling into a group significantly higher than density for April 26 but not significantly higher than May 30 or June 14. Unclipped plots were significantly different among dates ($F=14.9$, $df=5$, $p<0.0001$). Density data for April 26 and August 1 were not significantly different from each other, but were significantly lower than May 17, May 30, June 14, and August 30 which were not significantly different from each other.

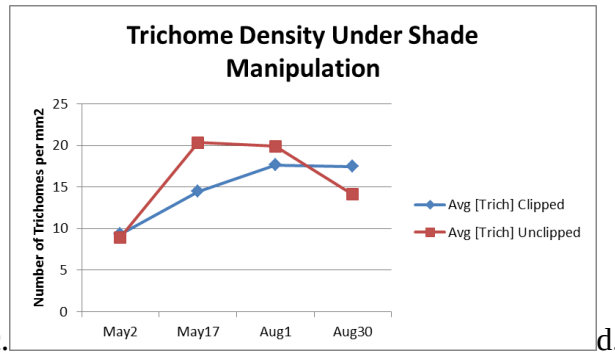
In 2009 data were collected from Lottie Bog (Fig. 5c) on May 2, May 17, August 1 and August 30. Trichome densities in clipped plots were significantly different with respect to date ($F=46.1$, $df=3$, $p<0.0001$). Trichome densities recorded for May 2 were significantly lower than those for May 17 which were significantly lower than August 1 and August 30 which were not significantly different from each other. In control plots there was a significant difference in trichome density with respect to dates ($F=73.7$, $df=3$, $p<0.0001$). May 2 was significantly lower

than May 17 and August 1 which were not significantly different from each other but were significantly higher than August 30.

A second field season was conducted in Lottie Bog in 2010 (Fig. 5d) with leaf samples being taken in April, May, June, July and August, each in the middle of the month. Trichome densities in clipped plots were significantly different with respect to date ($F=51.8$, $df=4$, $p<0.0001$). Trichome densities recorded for April, May and June were not significantly different from each other and were significantly lower than trichome densities recorded for July and August which were not significantly different from each other. In control plots there was a significant difference in trichome density with respect to dates ($F=12.8$, $df=4$, $p<0.0001$). May 2 was significantly lower than May 17 and August 1 which were not significantly different from each other but were significantly higher than August 30.

a. 

b. 

c. 

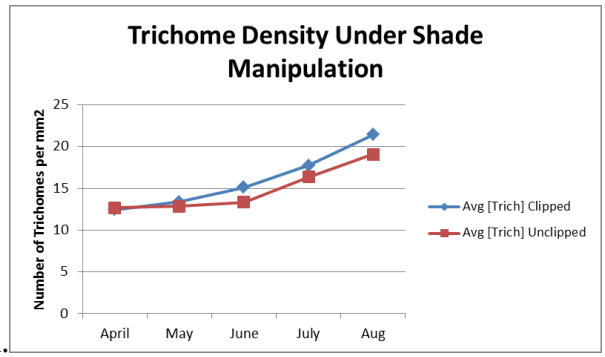
d. 

Figure 5a-d. Trichome density in clipped and unclipped plots in a. Big Bog in 2009, b.TNC Bog in in 2009, c. in Lottie Bog in 2009, and d. Lottie Bog in 2010

Discussion

The evolution of carnivory in plants in the Lower Gulf Coastal Plain occurred in habitats which are characterized as open, sunny habitats with sandy, loamy, generally acidic soils which are periodically saturated for a portion of the year (Folkerts 1982). These habitats are also subject to fire, which may occur as frequently as every one to five years (Folkerts 1982). In all carnivorous plants, the structures involved in carnivory are modified leaves, leaf parts or glands (Juniper et al. 1989). Carnivory allows plants in these habitats to derive nutrients from the capture and digestion of prey (Givnish 1989, Ellison and Gotelli 2001). A model proposed by Givnish et al. (1984) states that the benefit of carnivory in plants increases as a plant's ability to conduct photosynthesis increases. That is, carnivory is only beneficial when photosynthesis is not limited by other factors, such as light or water (Givnish et al.1984). In this study, I designed a set of experiments that would test the effects of varying light levels on the density of trichomes produced by *Drosera capillaris* as a measure of investment in carnivory and to determine the appropriateness of trichome density as a measure of investment.

I designed a greenhouse experiment in order to create a more controlled version of the experiment I conducted in the field. Unfortunately, in the greenhouse experiment no significant changes in trichome density were observed, either in the shaded or unshaded plants. This is likely due to a combination of exceptionally small sample size and high mortality of plants in the greenhouse. Plants were initially collected from a roadside population and kept in a growth chamber until the greenhouse space was prepared. During this time, it is likely that the plants

became acclimated to the mild conditions of the growth chamber, causing the move to the greenhouse to be too great a shock for them to survive. Only two of the initial twelve plants in the unshaded treatment survived to the fifth measurement and only one to the sixth measurement. However, if the fifth and sixth measurements are disregarded due to exceptionally small sample size and their stressed condition in the unshaded treatment, a trend of an increase in density in the unshaded treatment and a decrease in the shade treatment is evident, though it is not significant.

In the prey escape experiment there was no significant difference between treatment and control plants with regard to how many flies escaped in each trial. There are multiple potential causes of these results. First, the sample size in this experiment was very small, only 14 pairs of plants for the entire experiment. It is also quite likely that the fruit flies used were too large for *Drosera* to retain on its leaves. Prey body size thresholds have been determined for other species of carnivorous plants including *Drosera filiformis* (Gibson 1991). Arthropods commonly found on leaves collected in the field are collembolans, mites and other similarly small arthropods (Personal Observation). Also, the same set of fly cultures were used through all the trials and the cultures were visibly degraded by the final trials of the experiment. It is possible that over time, due to worsening conditions of the fly cultures, that the flies were weakened sufficiently to be retained by the leaves. This would explain the increased prey escape rates in the treatment plants in later trials.

While results for this experiment were not significant, I believe that trichome density is an appropriate physical character for measuring investment in carnivory. Zamora et al. (1998) used a variable they called “Mucilage secretion” in which the measured droplet size and stalked gland density were used to calculate mucilage volume secreted per unit of leaf surface.

However, droplet size was determined after the leaves were collected when evaporation probably compromised the diameter of mucilage droplets to some degree. Darwin (1875) noted that the amount of mucilage produced varies with relative humidity and was produced in response to prey items and varied depending on the item placed on the leaf. However, Zamora et al. (1998) along with studies conducted by Alcala and Dominguez (2003, 2005) showed that trichome density is a plastic character which changes in response to environmental cues. I would conclude from this information that trichome density is then a preferred character for measure of investment in carnivory as it is an easily quantifiable character which is not altered after collection.

Thoren et al. (2003) referenced this method by Zamora et al. (1998) and used a variable they called leaf stickiness. They found that decreasing light decreased investment in carnivory as measured by leaf stickiness (Thoren et al. 2003). Thoren et al. (2003) measured stickiness by placing filter paper on healthy leaves and attaching the filter paper to a dynamometer which was pulled up and a reading was recorded as the filter paper separated from the leaf. The size of the leaf, the density of the trichomes, the amount and viscosity of mucilage and the conditions of the room in which the leaves were tested in, would all play a part in this measurement and it is difficult to determine which factor is most important in investment in carnivory.

Alcalá and Domínguez (2005) determined allocation to carnivory-related structures in *Pinguicula moranensis* by calculating the density of trichomes from collected leaves. I used this method to determine investment in *Drosera* as it allowed me to avoid the confusion of ephemeral environmental conditions which are often encountered in measuring the viscosity or amount of mucus produced by these plants. I suggest that further study be conducted on the use of trichome

density as a suitable character for gauging investment in carnivory, perhaps an experiment which either utilizes a larger species of *Drosera* or a more appropriate sized prey item.

A concern in designing the field experiment was that the differences I might detect in trichome density would be due to differences in plant health due to excessive or insufficient light. In both field seasons, rosette diameter and number of leaves were recorded for each of 100 tagged plants of each treatment in each bog on each trip to the field. These data show that there was little to no difference in changes in adult plants between the two treatments over the course of either field season (Fig. 4a-d). This allowed me to have some assurance that any effects I detected in trichome density relating to treatment were not simply due to etiolation effects or changes in plant health due to treatment.

For my field experiment concerning the manipulation of available light on trichome density, my prediction was that increasing light available to *Drosera capillaris* would allow a greater investment in carnivory as measured by trichome density. For this to be supported, I would have expected my data to show similar beginning trichome densities in clipped versus unclipped plots at the beginning of each field season and significantly different trichome densities between clipped and unclipped plots at the end of the season (with densities in the clipped plots being higher).

In 2009 in Big Bog, clipped plots began with a significantly lower trichome density than unclipped plots. On the final day of treatment no significant difference between clipped and unclipped plots was evident, contrary to my prediction. Although I cannot explain the initial differences between treatment plots, the change shows an increase in trichome density in clipped plots by the end of the season so that this difference no longer existed. TNC Bog began with similar trichome densities between clipped and unclipped plots. On the final day of treatment

recorded trichome density of clipped plots was no significantly greater than that of unclipped plots, contrary to my prediction. In Lottie Bog in 2009, trichome density was not significantly different between clipped and unclipped plots on the initial day of treatment but was significantly different on the final day of treatment, with clipped plots having greater recorded trichome densities, in support my initial hypothesis.

Lottie Bog was the only bog utilized in 2010. Clipped and unclipped plots were not significantly different from each other on the first day of treatment. At the end of the treatment, trichome density in clipped plots was not significantly different from that of the unclipped plots, but average trichome densities for clipped plots were higher, on average, than in unclipped plots, (if not significantly so).

Treatments did not have the predicted effect in Big Bog or TNC Bog in 2009 or in Lottie Bog in 2010. This could be due to a number of factors. An assumption made in the initial hypothesis is that carnivory becomes more adaptive in habitats where light and water are not limited (Givnish et al. 1984). It is possible that water was somehow limited in these two bogs, which would prevent me from observing the desired effect. Multiple factors can affect the amount of water available to these plants in the soil. Natural factors such as decreased rainfall or lack of water available in the soil due to high in ground competition from surrounding plants for water are possibilities. In my clipped treatment the underground portions of shading herbaceous plants were left intact so this form of competition was not accounted for. Also, all three bogs are distantly bordered by pasture land, runoff from which could affect nutrient levels. Lottie Bog is bordered nearby by a paved road and Big Bog and TNC Bog by dirt roads, all of which can affect the hydrology of the soil, changing the amount of water available to the plants. If water

was somehow limited in Big Bog or TNC Bog, then increasing available light would not be expected to increase a plant's photosynthetic potential.

Another very likely factor is the amount of overstory and understory shade. Big Bog was a very open bog which has been well-managed for 14 years. This means that there is little overstory shade to contend with, that shading herbaceous growth was not overly dense, and there was not a great deal of standing dead herbaceous material. In other words, it is possible that the plants in Big Bog already existed in a relatively optimal state where photosynthesis was not being limitied by the amount of available light and so my treatment would have no significant effect. TNC Bog, on the other hand, was a very overgrown, shrubby bog with a relatively high amount of overstory shade. While the difference in relative light availability I made with my treatment in TNC Bog was measurably significantly different, it was not so drastic a difference as that exhibited in Big Bog or Lottie Bog (Fig. 2). It is possible that there is a threshold of change in available light that must be reached before plants will expend the energy to acclimate to a change in light levels.

The data collected in Lottie Bog in 2009 show the expected trend. Plants in both clipped and unclipped plots began with similar trichome densities and had significantly different trichome densities on the final day of treatment. Plants in the clipped plots exhibited a significant increase in trichome density, while plants which had less available light showed no significant change in trichome density. The field season ended with clipped plots having significantly higher trichome densities than unclipped plots.

There are a couple of potential explanations for why Lottie Bog produced the expected results in 2009 but did not produce the expected results in 2010. Hydrology is a possibility. The Mobile weather station recorded 69.99 inches of rainfall in 2009, but in 2010 only 48.17. Plants

in 2010 were visibly stressed due to the dry conditions (Personal Observation). It is possible that available moisture was a limiting factor in 2010 in which case I would not expect to see the predicted results. Another possibility, though less likely, is that in the 2010 season, the amount of herbaceous vegetation and standing dead vegetation had finally reached a density that affected the *Drosera capillaris* in the initial stages of growth to the point that even with significant increases in light availability later in the season, the plants were simply not healthy enough to make adjustment for the added light.

In retrospect, even with a lack of significance in the 2010 field season, I believe that Lottie Bog was the ideal bog in which to conduct this experiment. Since fire had been excluded from Lottie Bog for at least 4 years prior to the beginning of this study, the shading herbaceous growth and standing dead vegetation had become relatively thick on the ground. However, the bog still maintained an open, sunny aspect above the herbaceous growth so the difference created by the removal of shading herbaceous growth and standing dead vegetation mimicked the fire that the bog had been lacking by removing the above ground competition for *Drosera capillaris*. This created the drastic change in available light that I believe I simply was not able to create in TNC Bog, and is why Lottie Bog produced the results predicted by my hypothesis.

The effect of variation of environmental conditions on a single population within a single growing season had not, before now, been observed. Alcalá and Domínguez (2005) found that, in *Pinguicula moranensis*, in populations which occur in naturally different environments (including shaded and sunny), trichome density, among other factors, varied among populations studied, but raised the question as to whether these carnivorous traits are the result of genetic differences among populations or of phenotypic plasticity. This is a question that my experimental design attempted to address. By changing the amount of light available to some

plants within a population, both in the field and in the lab, I attempted to discern whether in *Drosera,* and in these specific bogs these plants can respond to changes in their environment both within a given season and within a specific individual.

The results for Big Bog and TNC Bog in the 2009 field season show that there is more going on in these bogs than was accounted for by these experiments. The highly variable results between bogs suggest that there is, first of all, great variation in bog habitat, even those that were once part of the same bog system. Elevation, tree cover, fire regime, and amount of development are all important factors in how these plants grow and thrive in their environment. However, in results obtained from Lottie Bog, by changing the amount of light available to some plants within a population, I was able to demonstrate, at least in Lottie Bog in 2009, that *Drosera* has the potential to respond to changes in the environment within a given season and population through alteration of investment in carnivory as measured by trichome density. The model proposed by Givnish et al (1984) is only partially supported by recent research including this study. Others (Ellison 2006) have suggested alternate explanations for the patterns of carnivory in plants seen today, including phylogenetic constraints and environmental stress.

Drosera capillaris is only a small part of a large, fragile and unique ecosystem. The longleaf pine habitats of the Lower Gulf Coastal Plain, of which the wet pine savannahs are part, has been disappearing rapidly over the past two centuries (Folkerts 1982 and Kush et al. 2004). The more we study these habitats and the more we come to know about how they work and how they are affected by changes to the environment, either natural or artificial, the more effective we will become in conserving and restoring them.

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