PREDATION AND LIGHT AS DETERMINANTS OF VEGETATIVE GROWTH AND REPRODUCTIVE SUCCESS IN THE CARNIVOROUS PITCHER PLANT *SARRACENIA PURPUREA*

by

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Title: Predation and Light as Determinants of Vegetative Growth and Reproductive Success in the Carnivorous Pitcher Plant, *Sarracenia purpurea*

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Primary Thesis Advisors

The broad ecological range and population of *Sarracenia purpurea* are being reduced due to climate change and human encroachment. These iconic plants rely on both photosynthesis and carnivory, however the relationship between these two factors and their individual effect on growth and reproduction is unknown. With the constant threat that these fragile plants are under, it is very important that conservation efforts by plant sanctuaries, reserves, and national parks are provided with the most accurate data to implement updated care guidelines and ensure the longevity and protection of carnivorous plants.

My research exposes plants to bright or dim light while being fed or starved, and experiments were run in real environmental time, conducted over all four seasons. Plants were held in an environmentally controlled room programmed to provide daily annual light and temperature of the Gulf Coast of the Florida Panhandle and Alabama. Independent weekly censuses were conducted over the course of a year, as well as data collection of leaf widths, volumes, meristems, flowers, dry masses, and anthocyanin levels. This large data collection and calibration period provided the basis for which the independent effects of light and predation on both growth and reproductive successes could be resolved.

My research findings show that reproductive success of *S. purpurea* is promoted by access to adequate and non-obstructed light, where they are protected from UV damage by light-induced anthocyanins, whereas plant vegetative production is determined by access to prey.

Acknowledgements

I would like to thank Bill Bradshaw and Chris Holzapfel for their help during this entire thesis process. I am eternally grateful for everything you both have helped me to accomplish. I would also like to thank Daphne Gallagher for supporting me, both academically and personally throughout my time at CHC, and Lindsay Hinkle for her trust and flexibility during this whole process. Additionally, I would like to extend my thanks to my family for funding my education, pushing me forward, and supporting me through this difficult educational journey for which I am infinitely grateful. Thank you to Anna Janke, who cared for these plants in preparation for this project and for teaching me everything about the lab and these wonderful plants. Lastly, I would like to thank my brother Lukas, who helped me out immensely during this last year despite both of us being extremely busy. Without you all, this thesis would not exist.

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Introduction

Plants typically rely on photosynthesis as a source of energy. Some plants engage in carnivory as well as photosynthesis. Pitcher plants, whose leaves develop into a water-holding cup or "pitcher" are the most common form of carnivorous plant and are native to Australia, Indo-Malaysia, North America, and South America (Brewer and Schlauer, 2018). In the Americas, the most wide-spread belong to the Family Sarraceniaceae, consisting of three genera: *Darlingtonia*, restricted to Oregon and northern California, *Heliamphora*, restricted to the tepuis of Venezuela and Guiana, and *Sarracenia*, found in North America (Brewer and Schlauer, 2018). Of the latter, *Sarracenia purpurea* is the most geographically wide-spread, extending from the Gulf Coast, northwards in and east of the Appalachians, and then north and west of the maximum extent of the Laurentide Ice Sheet, ranging in Canada from Labrador to at least Saskatchewan (Fig. 1, McDaniel, 1971). These plants are found in wet, humid, and sunny locations like the Gulf Coast (Givnish et al., 1984).

In *S. purpurea*, the hood above the water-filled leaf is covered in fine downward pointing hairs enhancing prey capture and the slick cuticle in the upper portion of the leaf prevents prey from escaping (Płachno and Muravnik, 2018). Bacteria present in the pitcher liquor, break down the insects into nutrients for plant growth and development (Bradshaw and Creelman, 1984). The most common source of prey for *S. purpurea* are ants; but, field research conducted by Newell and Nastase (1998) indicates that there is a low prey-capture efficiency of about 0.37%.

Sarracenia purpurea have only been found to grow where it is wet, sunny, and the soil is lacking in nutrients (Givnish et al., 1984). Since bogs receive most of their

water due to rainfall as compared to runoffs or springs, these ecosystems lack vital nutrients, especially nitrogen (US EPA, 2015), which is essential for plant growth (Novoa and Loomis, 1981). In North America, there are many national and state parks that are dedicated to the preservation of this rare and endangered species. Human encroachment and climate change pose the biggest threats to carnivorous plants, particularly due to their fragile habitats that can easily be disturbed by dry spells, excessive heat, and run-off from adjacent agriculture that serve as fertilizer for competing plants. In northern Florida, pitcher plants are typically shaded by tall wire grass and are partially screened from direct sunlight (Fig. 2). Carnivory is generally assumed to help compensate for the environmental disadvantage of living in nutrient-poor, wet habitats. Prey captured by the pitcher plant also serve as the nutrient base for a diverse aquatic community of mosquitoes, midges, flies, mites, rotifers, protozoa, and bacteria (Bradshaw, 1983).

The natural magenta coloring expressed in *S. purpurea* derives from the pigment anthocyanin (Sheridan and Griesbach, 2001). This flavonoid is a polyphenolic secondary metabolite, and is commonly found in berries such as grapes, blueberries, blackberries, blackcurrants, strawberries, and bilberries, as well as in red/purple vegetables, flowers, and leaves (Wallace and Giusti, 2015). Anthocyanins function to attract pollinators and protect chloroplasts from the photoinhibitory and photooxidative effects of bright light by absorbing high energy UV-B rays (280-315 nm; Gould, 2004). Anthocyanin production is based on a regulatory gene in *S. purpurea,* and when the recessive allele of this gene is expressed, a new anthocyanin-free phenotype is observed leading to expression of leaves and flowers that are entirely green (Sheridan et al.,

1997). The absence of anthocyanin is not fatal as the green phenotype persists in some natural populations. Schaefer & Ruxton (2007) proposed that anthocyanins enhanced prey attraction; but, Bennett and Ellison (2009) conclude that prey attraction is strictly mediated by the nectar secreted by plant leaves. From these more recent results, anthocyanins play no role in prey capture, and only function as a UV screen to filter harmful UV rays from damaging cellular tissues (Gould, 2004).

Experiments previously conducted by Bradshaw and Creelman, (1984) showed that *S. purpurea* leaves take up ammonia and carbon dioxide from their pitcher-pot liquor and in turn infuse oxygen back into the pitcher-pot liquor. These experiments highlight the significance of mosquitos like *Wyeomyia,* in potentially accelerating the plant's nutrient uptake. Bradshaw and Creelman (1984) also show that feeding with mealworms is experimentally appropriate; consequently, my research will also use mealworms as "prey" for *S. purpurea*.

Research into the effects of carnivory in *S. purpurea* without the symbiotic relationship with other organisms has not been conducted, although it is widely believed that the evolution of carnivory in carnivorous plants was not due to the mutual beneficial relationship it creates with the surrounding ecosystem (Ellison et al., 2001). The specific benefits of carnivory are that *S. purpurea* gain nutrients that are not found in their environment from prey that are used for plant growth and development consistent with a cost-benefit model (Ellison & Gotelli, 2009). Two of the three genera in the family Sarraceniaceae, *Darlingtonia* and *Heliamphora*, receive up to 80% of their nitrogen from digested insect prey (Schulze et al., 1997), whereas *Sarracenia* receive around 10% (Chapin et al., 1995).

The fascinating and unusual *Sarracenia purpurea* is featured in many state parks and preserves (Table 1) and was chosen by Queen Victoria as the provincial flower of Newfoundland (Gouvernement Du Canada, 2017). As the effects of human encroachment and climate change grow increasingly more severe, the managed preservation of *S. purpurea* in both northern bogs and southern costal savannas of North America is becoming more important. To protect and foster these plants, management practices should be targeted at both plant vegetative growth as well as reproductive success.

My thesis encompasses 15 months of research into the effects of carnivory on *S. purpurea* sexual reproductive success (flowering) and vegetative growth under different light conditions. Experiments are run real-time through all four seasons, accounting for the dormant as well as the reproductive period. My thesis includes prior work conducted by previous undergraduate researchers on the same plants used in my research, providing the experimental background for my experiments.

Materials and Methods

Approach

My basic approach was to expose plants to bright or dim light while being fed or starved, making four treatments with multiple plants per treatment. Experiments were run in real-time in an environmentally controlled room programmed to provide daily annual light and temperature of the Gulf Coast of the Florida Panhandle and Alabama, the origin of my experimental plants.

Plant origins and lab maintenance

Plants were originally either from the Blackwater Fisheries Research and Development Center, near Holt, FL, in 1973, where they were rescued from the excavate of a new fish pond, or collected as seed from roadside ditches along the Gulf Coast in the 1970s and 1980s. They were maintained in the lab and augmented by fragmentation of the rhizomes or grown from seed after cross pollination. Plants occasionally became infected with scale, which were resistant to bio-degradable, topical pesticides including insecticidal soap (Saferbrand.com) and malathion (Spectracide.com). Consequently, infested plants were cycled through the University greenhouse, where they were treated with systemic Orthene (AMVAC.com). Treatment consisted of three applications, a week apart, followed by a year in the greenhouse to clear the Orthene, before returning to the lab.

Background plant history 2018-2021

2018-2019. In spring of 2018, Orthene-treated plants were transferred back to the lab into a computer-controlled climate room programmed to provide real-time daily and annual light and temperature cycles of 30°N (Fig. 3 & 4). Potted plants were placed in 88.6x42.2x15.6cm LWD tubs (Sterilite, True Value Hardware) and bottom watered with tap water every 48-72h.

2019-2020. Plants were ranked by leaf size from smallest to largest of 98 plants. Consecutive pairs were assigned individual feeding treatment by flipping a coin and numbered 1-49 for fed plants and 51-98 for starved plants. Fed plants were provided haphazardly a size-specific diet of freeze-dried adult *Drosophila melanogaster* from April (subjective time) until plants ceased producing leaves in the subjective winter, 2020.

2020-2021. Plants were maintained from spring 2020 (Fig. 5) through their entire year without food. Number of meristems per plant was determined and recorded. Plants were censused weekly, leaves on each plant labeled consecutively alphabetically; width at widest point perpendicular to the keel, and volume of each leaf were recorded when the leaf had become firm and leathery to the touch. Dates of flower budding and flower opening were also recorded. During 2019-2021, 22 plants died or were removed because they acquired scale, leaving a total of 66 experimental plants. These plants provided the baseline population for comparing subsequent effects of food and light intensity on vegetative growth and reproduction (flowering) in 2021-2022.

Light and temperature

Light and temperature were designed to replicate the daily and seasonal variation experienced along the Gulf Coast (Bradshaw et al. 2004). The environment was managed with computer-driven, custom designed Siemens controls (https://new.siemens.com/deign/controllers) in a large (2.4 x 4.9m) room with constant mixing by four fans to minimize hot or cold spots. Relative humidity was held constant at 85% and light and temperature were varied daily and annually as shown in Figure 3. Actual leaf temperatures (Fig. 4) were recorded by two Watchdog A110 data loggers (Spectrum Technologies, https://www.specmeters.com/weather-monitoring/data-loggers/a-series-loggers/).

Plant illumination consisted of eight, Phillips High Performance fluorescent lamps (F32T8: 3100 lumen, color temperature 4200K per lamp), 45-56cm above the leaves (https://www.usa.lighting.phillips.com). In experiments where illumination was varied, "Bright" illumination consisted of the same eight lamps; "Dim" illumination consisted of two of the same lamps. Distance from the lamps to the plants was held constant in both bright and dim treatments.

Plant Metrics

Meristem, leaf production, and flower number. Pitcher-plant leaves originate from an apical growth zone, or meristem. Some plants are also prone to adding new meristems to the growth zone, so that leaves can arise from multiple meristems in a given plant. To determine whether plants with more meristems produce a greater number of leaves, I scored each plant as to the number of meristems on that plant,

permitting analysis of vegetative growth and flowering on a whole-plant or per-meristem basis.

Leaf width, volume, and dry mass relationships. When a leaf had opened and had become firm and "leathery" to the touch, I measured leaf width at its widest point perpendicular to the keel with a digital caliper (OriginCal, Amazon.com), consecutively numbered each leaf on a plant with an indelible, spirit-based pen. At a later date, I assessed leaf volume by filling a leaf to overflowing, decanting the fluid, and measuring the decanted fluid in a graduated cylinder to the nearest 0.5mL. In addition, I measured the volume of 20 leaves ranging from small to large, cut them from the plant at the narrowest zone of the petiole, and dried them to constant mass in a desiccator using Drierite as a desiccant (fishersci.com). Dry mass data was collected using a vintage Christian Becker Chainomatic balance.

Experiments 2021-2022

Light & food. To start my experiments, the 66 plants were ranked by size into sequential cohorts of four plants each. Within each cohort, plants were randomly assigned light and food treatment using a deck of cards for randomization. Within the experiment there are four experimental groups: bright light + starved (BS), bright light + fed (BF), dim light + starved (DS), and dim light + fed (DF), leaving 13-14 plants per treatment. Fed leaves were fed twice: two weeks after opening, when they had become firm to the touch, and then again two weeks later. I used mealworms (PetSmart

"medium" size) as prey with the amount of mealworm fed to a leaf dependent on the size (width) of the individual leaf.

I first determined the maximum amount of mealworm to feed a leaf that also avoided over-saturating the leaf, leading to its decay. Plants were fed and the liquor was checked for two weeks. My tests consistently showed overfed leaves, where the pitcher liquor would turn cloudy and rancid. This inability of a leaf to clear its contents within two weeks, resulted in leaves rotting and dying. After three rounds of testing (Table SI 1), I determined the maximum amount of mealworm that leaves of different sizes were able to digest and clear within two weeks. Original testing used leaf volume as a determinant for dietary conditions; however, I used the correlation between leaf volume and leaf width from 2020-2021 (Fig. 6) to create a more practical feeding protocol based on width (Table 2). I fed plants once when the leaf opened and was firm to the touch, and again 2 weeks later. This protocol ensured that the pitcher liquor was fully saturated with prey, but still able to clear the water by the end of the first and two-week period.

Vegetative growth. I conducted weekly censuses when new leaves were recorded and numbered with a sprit marker as a leaf initially opened. As leaves reached maturity (4+ weeks since initial opening), I recorded the volume and width perpendicular to the keel of each individual leaf. Previously, a plant's vegetative growth had been defined by the number of leaves produced per meristem. In addition, I quantified total vegetative effort as the number of leaves produced per year multiplied by the average leaf volume of the plant. This metric integrates the number of leaves and

the size of those leaves, providing a more accurate value for the quantity of leaf mass produced per plant. Herein, I experimentally test whether there is a direct relationship between the year 2020's bright starved plants with plants under different conditions in 2021: bright starved (BS), bright fed (BF), dim starved (DS), and dim fed (DF). I expected that there is a direct relationship between last year's total vegetive effort and the current year's experimental plants. Consequently, I regressed this year's on last year's total vegetative growth (Fig. 7) and tested for differences among light and food treatments in the residuals using two-way ANOVA with replication.

Reproduction. I censused the plants weekly, recording the number and dates of flower opening. The first bud was recorded on April 6th, and the first plant flowered on April 20th (subjective time). The number of flowering plants was insufficient to meet the assumptions of a Chi-squared test. I therefore used Fisher's Exact Test (2-tailed) to test for differences in fed vs. starved and bright vs. dim light treatments separately (Fig. 8).

Anthocyanin. All plants were removed from their shelves and placed on a table with individual labels covered. There were 53 plants in total, as plants numbered 61, 60, 45, and 6 were omitted due to their small leaves and inability to rank them accurately, leaving 49 plants. I selected the plant with the reddest leaves (color rank 49); from the remaining 48 plants, I selected the next reddest plant (color rank 48), and so on, to the greenest plant (color rank 1). The final sample size consisting of 49 plants, forming a 7x7 red-green gradient (Fig. 9). I then decoded the labels and assigned a red rank 1-49

increasing in anthocyanin to each plant (Table 3). I returned plants to their original tub positions on the plant shelf within two hours.

Using red ranks for each light/temperature treatment (Table 4), I applied the Scheirer-Ray-Hare test (Sokal and Rohlf, 1995, Box 13.12) for ranked data in lieu of a parametric ANOVA. For this test, each treatment (bright fed, bright starved, dim fed, dim starved) must have equal sample sizes. Since the treatment bright-starved had 13 plants included in this gradient, the median red rank in this treatment was omitted from analysis (plant 18 with a red rank of #32), leaving 48 plants with 12 plants per treatment for a balanced design.

Statistical methods.

One- and two-way ANOVAs, regression, correlation, and calculation of the Scheirer-Ray-Hare test used the Excel Analysis Toolpak in Microsoft Office 16. ANCOVA used *JMP Start Statistics* (Sall et al. 2005). Fisher's exact test used VassarStats Frequency Data (http://vassarstats.net/), verified using the example in Box17.7 from Sokal and Rohlf (1995).

Results

Plant Metrics

Meristem, leaf production, and flower number

Plants with an increasing number of meristems produced more leaves and were not significantly affected by light or food treatments (Table 5). Consequently, subsequent analyses of vegetative growth and reproduction did not take number of meristems into account.

Leaf width, volume, & dry mass relationships

In 2020-2021, under bright lights with no food, leaf volume scaled as to the 2.053±0.038SE of leaf width (Fig. 6). The scaling coefficient differed from 3.0 ($t = 24.95$, $df = 541$, $P < 0.001$) but not from 2.0 ($t = 1.39$, $df = 541$, $P = 0.16$). In 2021-2022, with varying light and food, leaf volume scaled as to the 1.37±0.95SE of leaf width (Fig. 10). Residuals were greater in dim-starved leaves but did not differ from zero in the other three light/food treatments (Fig. 11).

Dry mass of leaves was linearly predicted from volume of intact leaves on plants (Fig. 12).

Light, food, and vegetative growth

Fed plants resulted in more total vegetative effort than starved plants, regardless of light conditions (Fig. 13).

Light, food, and reproduction

Bright light promoted reproduction (flowering) than dim light, regardless of food. (Fig. 8).

Light, food, and anthocyanin

The level of anthocyanin (indicated by red ranking) is higher in plants receiving bright rather than dim light, regardless of food (Fig. 14).

Discussion

Vegetative effort is correlated between years: plants that produce more/larger leaves one year are likely to produce more/larger leaves the next year. Total vegetative growth is promoted more by prey capture than light, while sexual reproduction (flowering) is promoted more by light than by prey capture (Figs. 7, 12). Investment in UV-protectant anthocyanins is also promoted by light and not prey capture (Fig. 8, 13). Together, these results are concordant with *S. purpurea*'s thriving in sunny portions of wet savannahs and boreal bogs (Givnish et al. 2018), despite increased, potentially harmful UV exposure from direct sunlight (Gould, 2004).

As the threat of climate change looms towards the irreversible and human encroachment on natural habitats expands, the quest for reliable ecological data becomes more important. Currently, members of the genus *Sarracenia* are threatened or endangered and are actively being protected by private or state funded national parks, plant sanctuaries, reserves, and conservations (Table 1).

National parks, as well as government funded conservations, are focused on the preservation and growth of many different types of *Sarracenia*. My study provides ways to improve managed populations of *S. purpurea*. The most direct course of action would be to enhance plant diversification and proliferation through sexual reproduction by exposure to bright light. Increased or sustained exposure to sunlight could be accomplished through removing surrounding brush and grass by controlled burns, or in sensitive surroundings, at least mechanical removal of shading shrubbery. This practice would be especially effective if sexual reproduction also resulted in seed dispersal throughout a managed wetland, either naturally or by human transport.

Conclusion

My research shows that reproductive success of *S. purpurea* is promoted by access to adequate and non-obstructed light, where they are protected from UV damage by light-induced anthocyanins, whereas plant vegetative production is determined by its access to prey. My work will benefit plant sanctuaries, reserves, and national parks with clear and updated solutions, to ensure carnivorous plant persistence. In spite of human encroachment and climate change-imposed stress on their fragile ecosystems, with responsible management we can prevent the extinction of these iconic plants, as well as associated species in threatened wetlands. °

Tables

Table 1: Collection of environmental conservation preserves that are currently protecting any genus of Sarraceniaceae

Name	Location
Brokenhead Ecological Preserve	Canterbury, MB, Canada
Coosa Bog Preserve	Cherokee County, AL.
Darlingtonia State Park	Florence, OR.
Gulf State Park Pitcher Plant Bog	Foley, AL.
Greater Lovell Land Trust	Lovell, ME.
Avalon Wilderness Reserve	Newfoundland, Canada
Gros Morne NP	Newfoundland, Canada
Splinter Hill Bog Preserve	Perdido, AL.
Joseph Pines Preserve	Sussex County, VA.
Cooter's Bog	Vernon Parish, LA.
Suitland Bog	Washington, DC.
Brunswick Nature Trail	Winnabow, NC.

Table 2: Finalized diet based off experimental results shown in Table SI 1. Volume was converted to width for ease of experimental

Width (mm)	Diet
0-10	0
10-19.99	0.25
20-29.99	0.5
30-39.99	0.5
40-49.99	1

Table 3: Plants indicated by plant number) organized from highest to lowest red-green ranking by decreasing level of anthocyanin (Table SI 2)

2	92	90	52	16	69	9
43	93	75	41	54	58	88
33	62	24	94	51	76	66
64	55	44	17	19	38	68
18	27	89	67	74	35	86
95	59	70	87	42	91	48
12	77	98	65	1	47	34

Table 4: Plant red ranking separated by treatments. Each column is organized from highest (49) to lowest (1) red ranking. BF, bright-fed; BS, bright-starved; DF, dim fed; DS, dim-starved.

BF	BS	DF	DS
47	49	33	31
46	48	22	29
45	44	19	24
42	43	18	20
38	41	16	17
36	40	15	14
35	37	11	12
32	34	10	8
30	28	9	5
27	26	7	4
25	23	6	3
13	21	2	1

Table 5: 2-way ANOVA of leaves per meristem with treatments light and food. (Table SI 3).

S of V	SS	df	MS	F	P-value
Light	6.72	1	6.72	1.08	0.3039
Food	**16.13**	**1**	**16.13**	**2.59**	**0.1141**
LxF	8.25	1	8.25	1.32	0.2563
Within	298.91	48	6.23		
Total	124400.44	77			

Figures

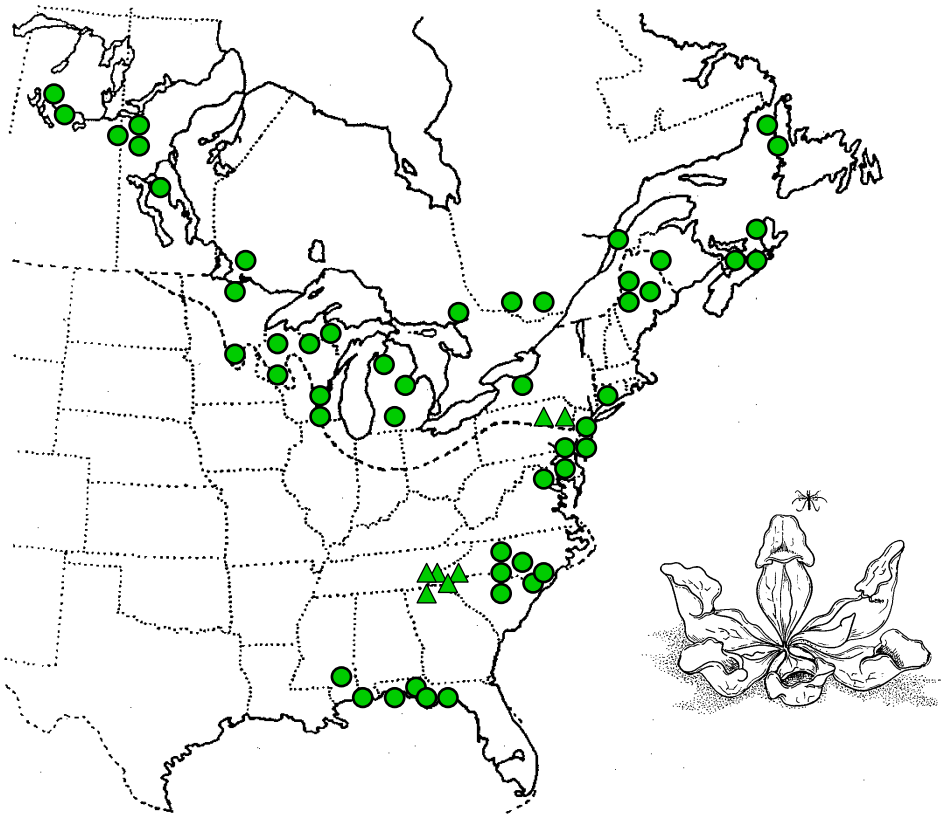

Figure 1: Distribution of S. purpurea in Eastern North America. Points indicate major mosquito collecting sites for the Brasdshaw- Holzapfel Lab.

Figure 2: Purple pitcher plant, *Sarracenia purpurea*, growing in Wilma Florida photographed by William Bradshaw and Christina Holzapfel. The plant is surrounded by tall wire grass which has been cut back to uncover the plant exposing pitcher pots full of rainwater.

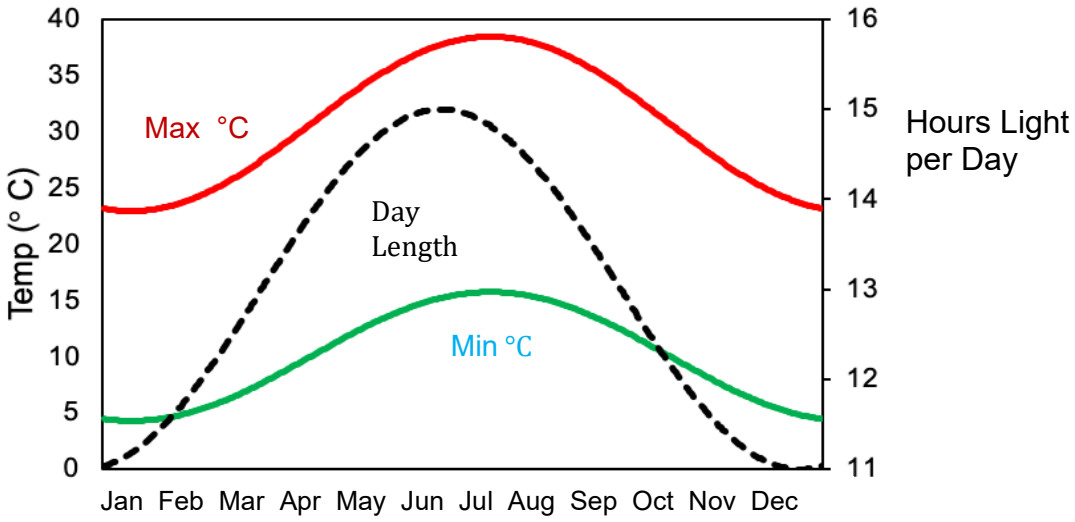

Figure 3: Programmed day lengths and daily and annual maximum and minimum room temperatures.

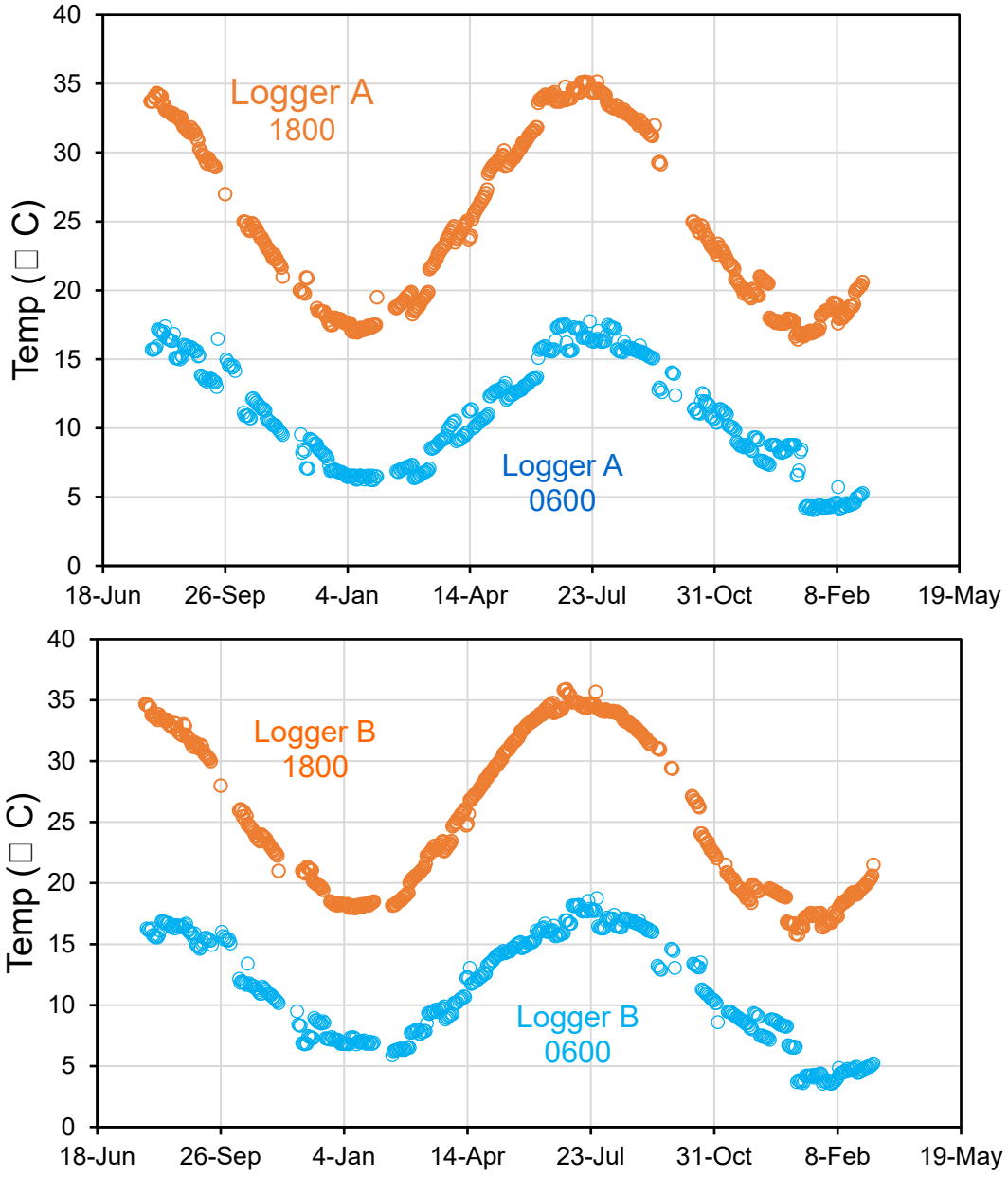

Figure 4: Actual leaf temperatures, 2020-2022 from WatchDog data loggers inside leaves on intact plants.

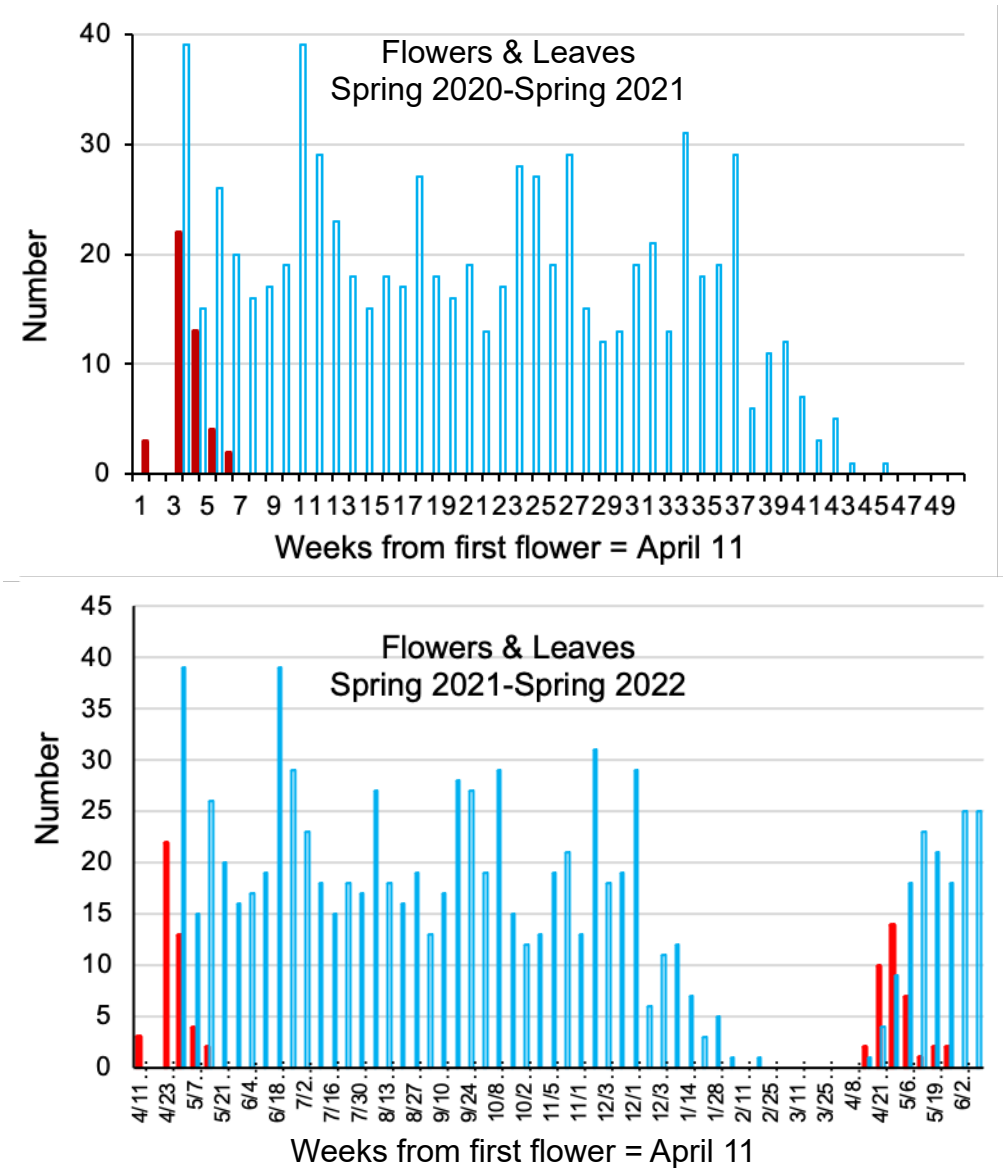

Figure 5: All flowers and leaves collected during 2020-2021 (top) and 2021-2022 (bottom). Flowers on 74 plants; leaves on 66 plants due to 8 plants lost to scale during the year. Red indicates flowers and blue indicates leaves. **Top**: Week 1 census on April 11, 2020; Week 50 census on March 16, 2021, subjective plant time. **Bottom:** Week 1 census on April 11, 2021; Week 63 census on June 2, 2022, subjective plant time.

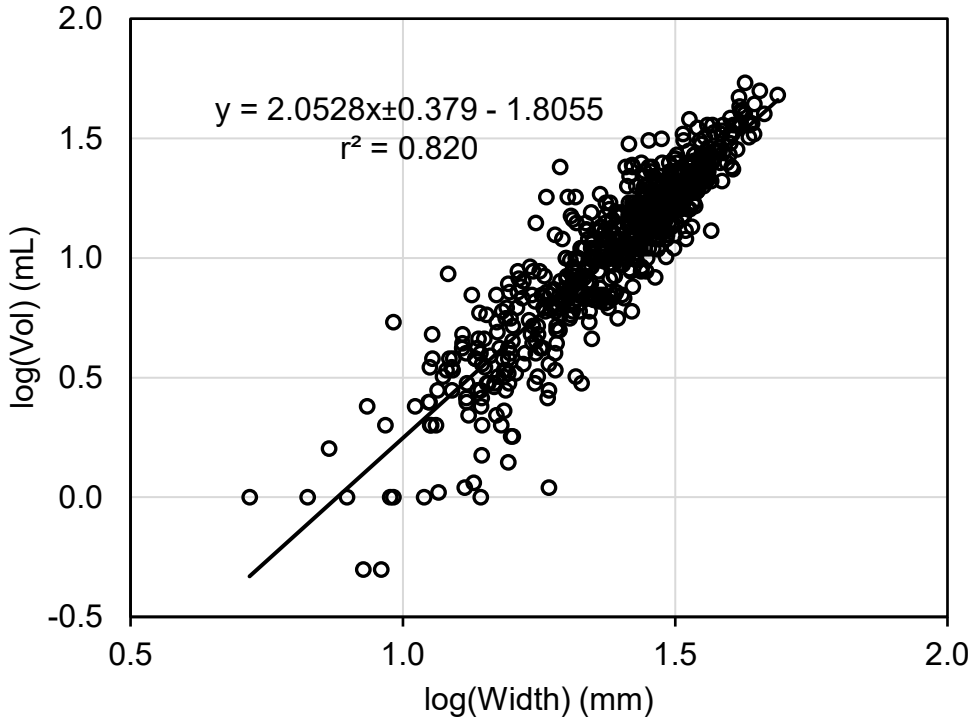

Figure 6: Maximum leaf width perpendicular to the keel as a predictor of leaf volume 2020-2021.

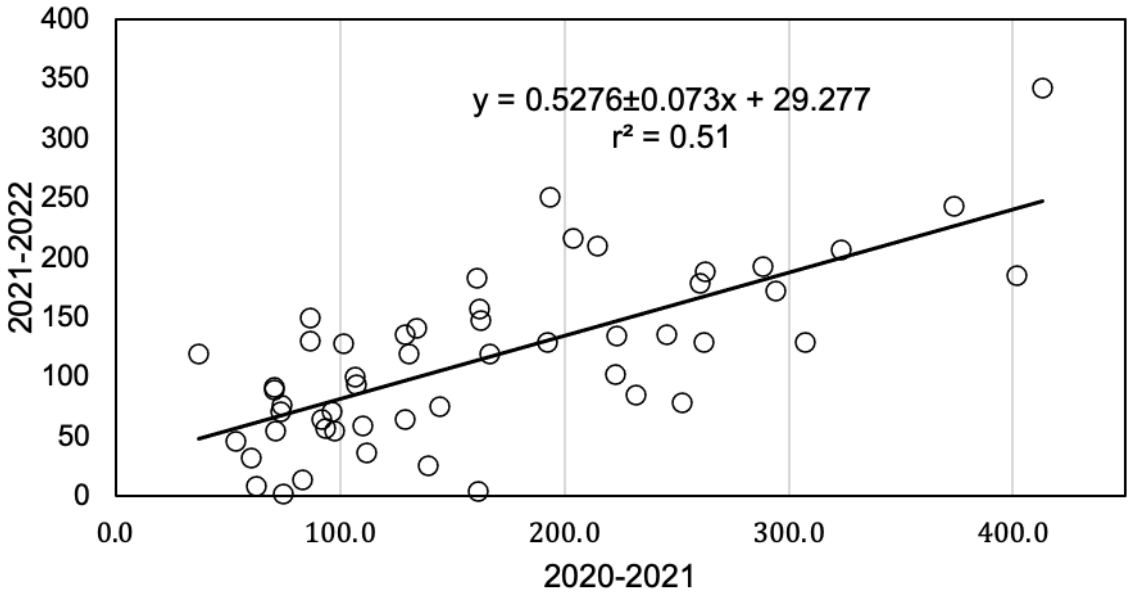

Figure 7: Liner regression of total vegetative effort in 2021-2022 on total vegetative effort in 2020-2021. Does not account for different treatments (BF, DF, DS) during the 2021-2022 experimental year (Table SI 4).

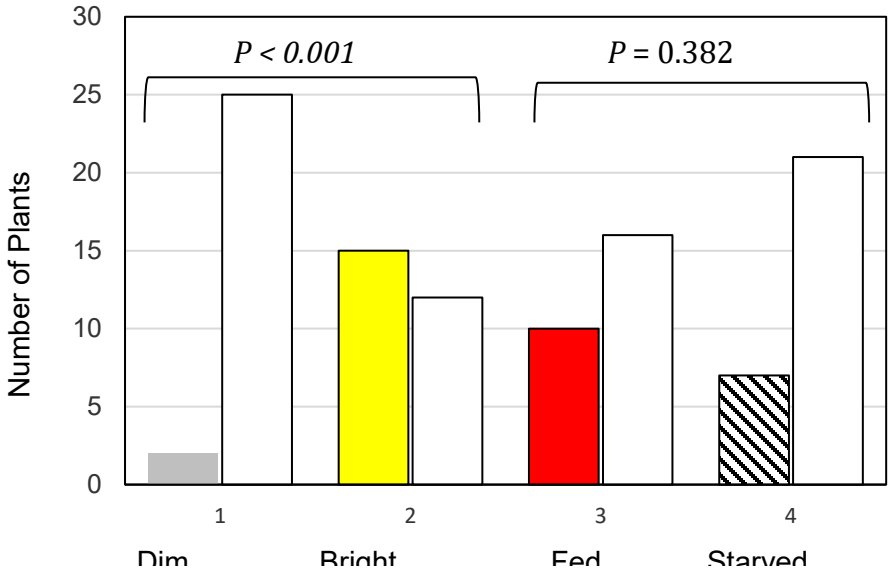

Figure 8: Effect of light and food on reproduction. Within each treatment, the filled bar indicates number flowering and the empty bar the number not flowering in 2022. *P*-values from Fisher's exact test, 2-tailed (Table SI 5)

Figure 9: Red (upper left) to green (lower right) rankings of 49 plants.

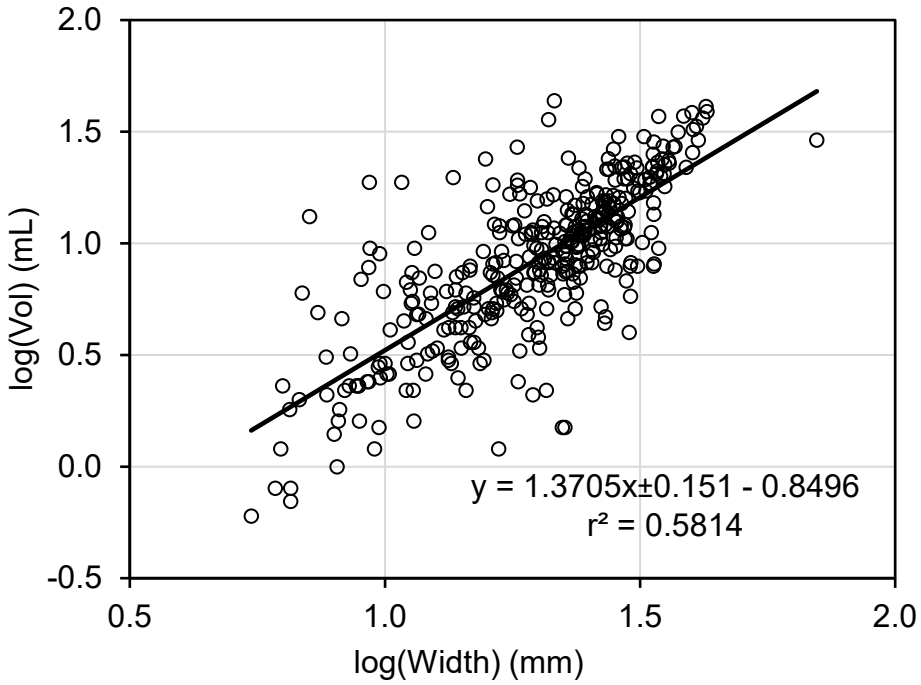

Figure 10: Maximum leaf width perpendicular to the keel as a predictor of leaf volume for the year 2021-2022.

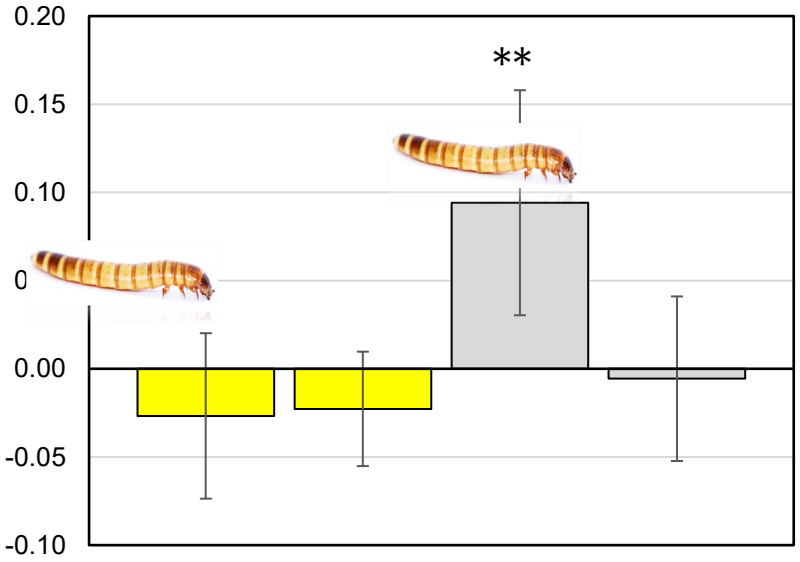

Figure 11: Effect of food and light on residuals from regression of leaf width on leaf volume. Light: bright, yellow; dim, gray. Fed, mealworm; starved, no mealworm. Error bars represent ±2SE. **P = 0.004; other means not significantly different from zero (Table SI 6).

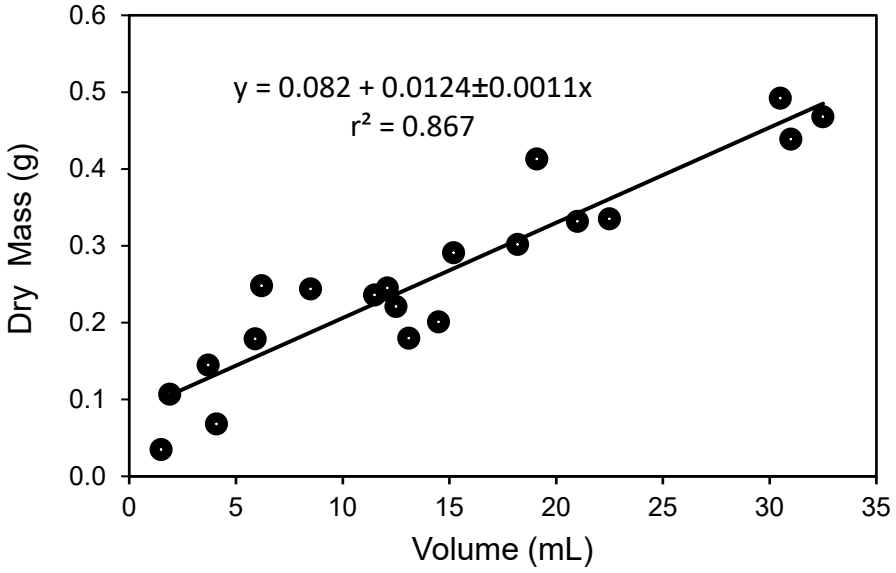

Figure 12: Leaf volume as a predictor of dry mass for the year 2021-2022.

Figure 13: Effect of light and food on year-long total leaf growth on a plant as measured by cumulative leaf volume from the year 2021-2022. Yellow, bright light; gray, dim light. Only feeding has a significant effect (P = 0.005, Table SI 7).

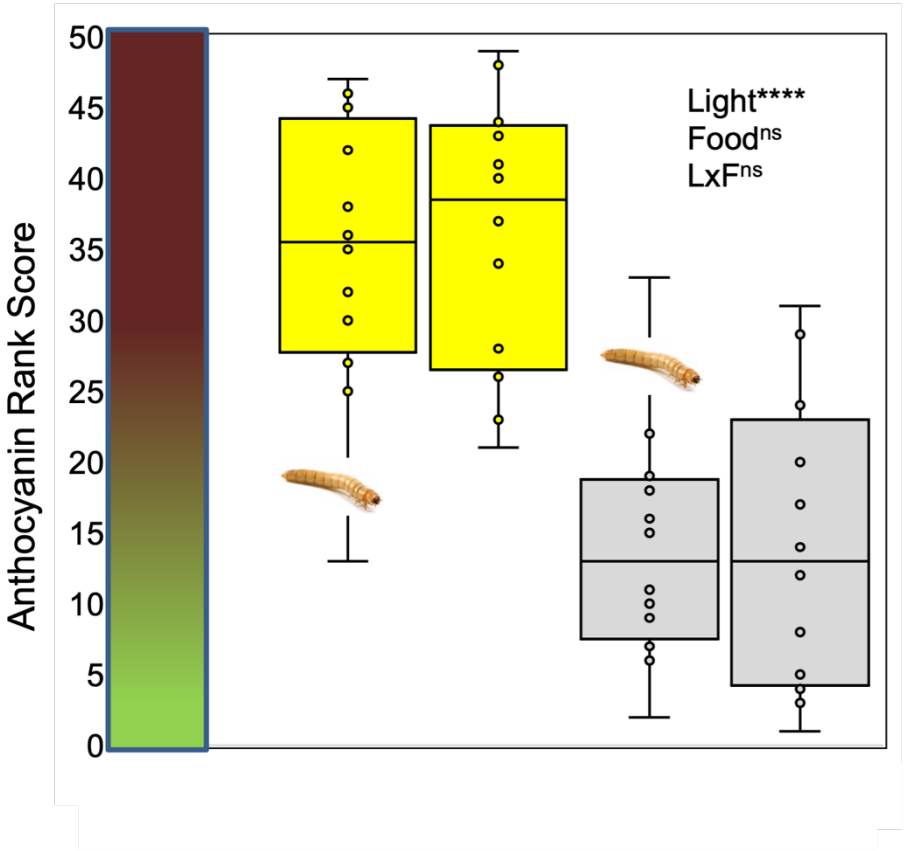

Figure 14: Median line and upper & lower quartile boxes of anthocyanin ranking in response to bright (yellow) or dim (gray) lighting and feeding (mealworm) or starved (no mealworm). Inset: results of Scheirer-Ray-Hare test: *** $P<0.001$; ns $P>0.05$.

Supplementary Information

Table SI 1: Experimental diets (fractions of mealworms) and corresponding leaf volumes for S. purpurea. Tests 1 and 2 resulted in cloudy pitcher liquor and rotting leaves. This led to a decreased amount of prey in Test 3 which was then implemented as the predation diet for my experiments.

Test 1:

Class	Vol (mL)	Diet
1	0-3.0	0.5
2	3.0-8.0	1.0
3	8.0-14.0	1.5
4	14.0-22.0	2.0
5	22.0-31.0	2.5

Test 2:

Class	Vol (mL)	Diet
1	0-3.0	0.25
2	3.0-8.0	0.5
3	8.0-14.0	1.0
4	14.0-22.0	1.5
5	22.0-31.0	2

Test 3:

Class	Vol (mL)	Diet
1	3.0-8.0	0.25
2	8.0-14.0	0.5
3	14.0-22.0	0.75
4	22.0-31.0	1

Table SI 2: Layout of highest to lowest red-green ranking by decreasing level of anthocyanin

49	47	44	40	35	29	22
48	45	41	36	30	23	16
46	42	37	31	24	17	11
43	38	32	25	18	12	7
39	33	26	19	13	8	4
34	27	20	14	9	5	2
28	21	15	10	6	3	1

Table SI 3: Actual number of leaves per meristem for each plant separated by treatment groups in preparation for analysis of variance.

Treatment	Fed	Starved
Dim		
	4	8.5
	5	5.5
	7	16
	4	6
	4	6
	4	7
	5	6.5
	9	6.5
	11	6
	4	11
	6	6.5
	6	8
	7	7.33
Bright		
	5	6
	9	6
	4	7
	2	8
	8	7.8
	11	1
	8	7
	8	7.67
	6	5.67
	2	8
	3	6
	4	4
	7	7

Table SI 4: Liner regression of total vegetative effort in 2021-2022 on total vegetative effort in 2020-2021.

Multiple R	0.7141
R Square	0.510
Adjusted R Square	0.5001
Standard Error	49.636
Observations	52

	df	SS	MS	F
Regression	1	128194.44	128194.44	52.03
Residual	50	123186.33	2463.73	
Total	51	251380.77		

	Coefficients	SE	t Stat	P-value
Intercept	29.277	13.918	2.104	0.0405
Slope	0.5276	0.0731	7.2134	2.78E-09

Table SI 5: Fisher's exact test, 2-tailed, for frequency of flowering in dim vs. bright light and fed vs. starved plants in

Treatment	Flower	No Flower	
Dim vs.	2	25	
Bright	15	12	$P = 0.0003$
Fed vs.	10	16	
Starved	7	21	$P = 0.3821$

Table SI 6: One-way ANOVA of residuals from 2021-2022 regression of leaf volume on leaf width, apportioned by light and food treatments.

Groups	Count	Sum	Average	Variance	SE	t	P
BF	110	-2.941	-0.027	0.061	0.023	1.1400	0.2568
BS	133	-3.024	-0.023	0.035	0.016	1.4018	0.1633
DF	67	6.308	0.094	0.068	0.032	2.9509	0.0044
DS	65	-0.366	-0.006	0.035	0.023	0.2415	0.8099

S of V	SS	df	MS	F	P-value	%TSS
Groups	0.7434	3	0.2478	5.1125	0.0018	3.96994
Within	17.9821	371	0.0485			
Total	18.7254	374				

Table SI 7: ANOVA of total, year-long leaf volume with light and food as treatments from the year 2021-2022.

S of V	*SS*	*df*	*MS*	*F*	*P*	*% TSS*
Light	7249.923	1	7249.923	3.624	0.063	5.885
Food	**17191.74**	**1**	**17191.736**	**8.594**	**0.005**	**13.956**
LxF	2723.688	1	2723.688	1.362	0.249	2.211
Within	96019.74	48	2000.411			
Total	123185.1	51				

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