Abstract:

The Purple Pitcher Plant, *Sarracenia purpurea*, is a carnivorous plant that lives in low-nitrogen environments such as bogs. This study looked at 50 pitcher plants in Mud Lake Bog, Michigan and used stable nitrogen isotope ratios and environmental and morphological factors to determine levels of prey-derived nitrogen in pitcher plant flowers and the factors that correlated with these nitrogen levels. It was hypothesized that flowering structures would be more reliant on insect-derived nitrogen than the pitchers themselves, and so expected to see an increase in prey-derived nitrogen in flowers. Significantly higher levels of insect-derived nitrogen were found in flowering structures compared to pitchers, with a mean in flowers of 73% prey-derived nitrogen. Pitcher size, color, soil temperature, surrounding biomass, number of flowers, and captured insect biomass all correlated with variation in prey-derived nitrogen values in the flowers. It was concluded that flowering structures preferentially received prey-derived nitrogen over pitchers, and that several environmental and morphological factors combine to affect the percentage of insect-derived nitrogen being used to produce these flowers.

Introduction:

Carnivorous plants are a group of over 600 species that have evolved a unique method of living in nitrogen-poor environments. In general, these plants have small, weak root systems and live in acidic environments with limited competition from other plants (Schnell 2002). For carnivorous plants to be successful in a habitat, there must be a balance of high light, high moisture, and low nutrient availability through litter (Benzing 2000). In most cases the evolutionary benefit of carnivorous structures is reflected in the trend that higher percentages of insect derived nitrogen correlate with more complex carnivorous structures (Ellison & Gotelli 2001).

The Purple Pitcher Plant, *Sarracenia purpurea*, is a carnivorous plant that uses a fluid-filled pitcher in order to attract and drown prey. However, this method of attracting prey has been found to have a low rate of capture, as often less than 1% of possible prey that enter the pitcher are actually captured (Newell & Nastase 1998). Furthermore, there is large variation in success rates of the individual pitchers in a plant. In fact, one study found that 8% of the pitchers on a plant catch 66% of the total prey biomass for the plant (Cresswell 1991). It has also been found that plants with newly opened pitchers will catch insects at a higher rate than plants without these new pitchers (Wolfe 1981).

Living in a nitrogen-limited environment, *Sarracenia purpurea* and other bog-dwelling plants are very sensitive to fluctuations in nitrogen levels. With increased CO$_2$ and nitrogen levels, like those predicted with increased global climactic change, *Sphagnum* bog species compositions are predicted to change, with even a decrease in the competitive success of the *Sphagnum* moss (Monique et al. 2001). One model predicts
an increased likelihood for extinction of the Purple Pitcher Plant with increased nitrogen deposition levels (Gotelli & Ellison 2002), displaying the plant’s nitrogen sensitivity. Like most carnivorous plants, the roots of *S. purpurea* are small, short-lived, and fragile (Adlassnig et al. 2005). The importance of these roots and the importance of carnivory can vary greatly based on environmental conditions. When nitrogen limitations are reduced, *S. purpurea* will change morphology to reduce carnivory as an effect of a reduced need for insect-derived nitrogen (Ellison & Gotelli 2002).

The morphology and traits of the pitchers of a *S. purpurea* plant will affect how many insects are captured by that plant, so the percentage of insect-derived nitrogen can vary greatly. It has been found that the greater the opening size of a pitcher, the more insect biomass it will collect (Cresswell 1993). Trends like this and others that may not have been quantified lead to a great variation in insect-derived nitrogen percentages. Observed percentages vary greatly, and can be as low as 10% (Chapin & Pastor 1995). However, few studies have looked at traits that affect the fitness or reproductive success of the Purple Pitcher Plant and what role insect-derived nitrogen plays in the production of reproductive organs.

This study focuses on the fitness of the Purple Pitcher Plant, *Sarracenia purpurea*, and how it is affected by phonological traits and insect-derived nitrogen levels. This study uses natural abundance stable isotope ratios to determine the source of nitrogen for the pitcher plants, as this process determines percentages of nitrogen from two sources to one sink. This is possible due to differences in percentages of the less abundant $^{15}$N compared to the more abundant $^{14}$N. As nitrogen proceeds up food chains, $^{15}$N will bioaccumulate in higher trophic levels, causing different levels to exhibit noticeably different $^{15}$N ratios (Minagawa & Wada, 1984). For carnivorous plants, this means that nitrogen derived from insects has a greatly different stable nitrogen isotope ratio, or $\delta^{15}$N, than nitrogen that is taken in through the roots. Therefore, this study uses $\delta^{15}$N values for insects, soil water, *S. purpurea* pitchers, and *S. purpurea* flowers to understand the role of insect-derived nitrogen in flowering structures and to better understand factors that relate to increased plant fitness. Using a sample of 50 Purple Pitcher Plants, this study looks at three questions regarding flowering structures and prey-derived nitrogen. The first question looks into what the average percentage of prey-derived nitrogen is in flowers and how that relates to leaves. Secondly, we will look at what environmental and morphological factors correlate with variation in prey-derived nitrogen levels in flowers. Finally, this study looks at which of these factors differ significantly between flowering and nonflowering pitcher plants. The study tests the hypotheses that insect-derived nitrogen is more heavily required in the production of flowers than leaves, that prey-derived nitrogen percentages in flowers correlate with several factors affecting both soil nitrogen availability and prey capture, and that flowering and nonflowering plants differ in several factors that affect the plants ability to overcome its nitrogen limitations. If this is true, we expect to find a statistical difference in means between insect-derived nitrogen in flowers and pitchers, correlations between traits and prey-derived nitrogen, and statistical differences between traits in flowering plants and the same traits in nonflowering plants.

**Methods:**

*Plant Selection*
The study site selected for this experiment was Mud Lake Bog (45°61’N 84°60’W) in northern Michigan, and data collection proceeded from early July to mid August, 2008. Mud Lake Bog is a Sphagnum dominated bog with pH ranging from around 3.0-5.7. At this site, 50 Purple Pitcher Plant individuals were selected for the study using several traits. 35 of the plants were flowering, while 15 were not. The plants were selected in order to get a sample representative of the variation present in pitcher color, amount of veination on the pitcher, pH, number of pitchers, number of flowers, amount of surrounding biomass, and the size of the pitchers. These individuals were then photographed and marked with a numbered flag.

At each flagged plant, one pitcher was chosen to be representative of the entire plant. This pitcher was chosen by looking at three traits: color, veination, and size. The pitcher with traits deemed closest to the plant average was then selected as the representative pitcher. Pitchers with holes or tears that affected their ability to hold water in the pitcher were excluded from selection.

Data Collection

Each S. purpurea individual surveyed had many on-site and in-lab measurements taken from it. The on-site measurements included number of pitchers, number of flowers, competition, pH, and soil temperature. The in-lab measurements included veination, color, insect abundance, pitcher symbionts, captured insects, hair density, hair length, pitcher δ15N, flower δ15N, insect δ15N, and moss δ15N.

The on-site measurements were collected at the same time for all plants to avoid discrepancies. Number of pitchers was counted by looking at only the pitchers produced within the past growing season, which was determined by texture and color, as older pitchers tend to be rough, brittle, and often somewhat brown in color. Unopened pitchers were not counted for this study. Number of flowers was counted by counting all opened, unopened, and dying flowers that were growing from the sampled individual. Competition from other plants was surveyed in a meter² area centered around the study pitcher plant. The plants that were measured were blueberry, leatherleaf, cranberry, orchids, sedges, tamarack, black spruce, bog laurel, bog holly, tag alder, and bog rosemary. Each pitcher plant was evaluated for total surrounding biomass in all of these categories on a scale from 1 to 10, with 10 being the most biomass. In general, only trees were counted to have biomass greater than 5. At intervals over the study period, pH and temperature were taken for each plant a total of three times. pH was measured on-site using a pH meter and a small plastic bottle. Water was obtained by pressing down on the surrounding moss until enough water emerged to fill the bottle, and then pH was taken in the bottle. The temperature of the water was also taken in order to calibrate pH readings. Soil temperature was measured for each plant using a thermocouple that was stuck 2 inches into the moss directly surrounding the plant.

In-lab measurements were taken using photographs of each plant and several samples taken at each plant. First, coloration and venation were quantified by removing the hood of each representative pitcher at the pitcher lip and taking a picture of it laid flat on graph paper. The hoods were then stored to measure hair density, length, and pitcher δ15N. Each picture was then analyzed for average color using Adobe Photoshop CS and a random-dot grid. The grid was placed over the computer screen and then the photograph was adjusted in size so that between 25 and 35 dots were on the image of the hood. The
eyedropper tool was then used to obtain R, G, and B values for all dots on the hood. Any dots that were located on a laceration on the hood or a spot with heavy reflective glare were excluded. R, G, and B values were then averaged for the entire hood. Venation was measured using the Image J program, where length of veins were measured by calibrating the pictures based on the graph paper, tracing the veins in the picture, and summing the total length.

Insect abundance was taken at each individual pitcher plant sampled using Aphid Whitefly Sticky traps made of nitrogen free petroleum wax (ARBICO-organics, Tucson, AZ). These sticky traps were placed at each location and left out between 72 and 96 hours. Sticky traps were then examined under a dissecting microscope to identify insects collected. These insects were then classified into the following categories: ???????.

Pitcher symbionts were measured by extracting the fluid from inside the representative pitcher for each sampled plant. Fluid was extracted using a plastic pipette with the tip removed. Once the fluid was extracted, the pitcher was refilled with water and then this was extracted again. This was done until insects were no longer appearing in the extract. Mosquito larvae (Wyeomyia smithii), fly larvae (Blaesoxipha fletcheri), midge larvae, mites, and rotifers were counted in-lab using a dissecting microscope and were also labeled as living or dead.

Hair length and density were measured using the pitcher hoods that were photographed for coloration and venation measurements. A circular cutout with a radius of 0.51 cm was taken from the center of the hood immediately above the pitcher rim and then was placed under a dissecting microscope. Ten adjacent hairs were selected randomly along a straight-edge then measured using a reticule eyepiece. The circular cutout was then divided into five segments. These segments were placed under a dissecting microscope and total number of hairs were counted a total of three times for each pitcher. This was then converted to hairs/cm² using the area of the circular cutout.

Finally, δ¹⁵N for insects, pitchers, flowers, and ground water was determined using a Elemental Analysis Combustion 2010 System and Thermo Finnigan Plus XP mass spectrometer. In order to prepare samples for the mass spectrometer, samples must be frozen at -80°C for several hours and then dried in an oven for one day. Then, the samples are ground into a powder using a mortar and pestle and then can be analyzed using the mass spectrometer. Insects were prepared for the mass spectrometer by separating insect parts from the filtered pitcher extracts. These insect remains were then dried in an oven at 55 °C, weighed, and then ground. Pitchers were prepared by drying and then grinding the hood taken from the representative pitcher. Flowers were sampled by taking the lowest petal from the northernmost flower of the pitcher plant, which were then prepared for the mass spectrometer. Finally, Moss samples were collected from underneath the pitcher plant and then were prepared for the mass spectrometer.

**Mixing Model**

The model for determining the percentage of insect-derived nitrogen follows from the assumption that insects and root uptake of nitrogen are the only significant source of nitrogen in the pitcher plant. With this assumption, we get the equation \( %N_I \times \delta^{15}N_I + %N_R \times \delta^{15}N_R = \delta^{15}N_P \) where \( %N_I \) is the percentage of insect-derived nitrogen, \( %N_R \) is the percentage of root-derived nitrogen, \( \delta^{15}N_I \) is the \( \delta^{15}N \) for insects, \( \delta^{15}N_R \) is the \( \delta^{15}N \) for root-derived nitrogen, and \( \delta^{15}N_P \) is the \( \delta^{15}N \) of the pitcher. We know that \( %N_I + %N_R = 1 \).
so substituting this into our original equation we get \( \%N_I \delta^{15}N_I + (1-\%N_I) \delta^{15}N_R = \delta^{15}N_P \) Which can be simply reduced to:

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\%N_I = (\delta^{15}N_P - \delta^{15}N_R)/(\delta^{15}N_I - \delta^{15}N_R).
\]

Results:

Prey-derived N in flowers as compared to leaves

It was hypothesized that flowers would have a high percent of prey-derived nitrogen and that this would be greater than the amount of prey-derived nitrogen in the leaves of the same plants. Measured values for flower prey-derived nitrogen ranged from 39.5% to 95.3%, with an average of 73.0%. This was compared to the average of 45.6% prey-derived nitrogen in corresponding leaves, which had a range of 4.8% to 82.9%. This produced a significant difference between levels of prey-derived nitrogen in flowers and corresponding pitchers (\( p<0.0005 \)) with an average difference of 27.4% (Appendix 1). Therefore, the mean prey-derived nitrogen in flowers (73.0%) was significantly higher than that of pitchers (45.6%).

Factors correlating to variation in prey nitrogen

Several of the environmental and morphological factors that might affect insect capture rates were shown to correlate with the variation in prey-derived nitrogen in flowers. Pitcher aperture, which ranged from 2.8 to 35.3 cm\(^2\) (Appendix 2), was shown to correlate negatively with the percentage of prey-derived nitrogen in flowers (\( p = 0.029, R = -0.374 \)). Also, pitcher length had a similar negative correlation (\( p = 0.004, R = -0.486 \)). These two factors show a trend towards higher percentages of flower prey-derived nitrogen in plants with smaller pitchers. Color was also shown to have a significant effect on the percentage of prey-derived nitrogen in flowers. G values correlated with prey-derived nitrogen (\( p = 0.022, R = -0.425 \)) as did the R:G ratio (\( p = 0.007, R = 0.489 \)). This correlation with the R:G ratio (Figure 1) can be interpreted to mean that plants with red pitchers will correspond to higher prey-nitrogen percentages than those with greener pitchers. Neither hair density, hair length, nor pitcher symbionts showed any correlation with flower prey-derived nitrogen (Appendix 2).

Factors affecting nitrogen availability in the soil also showed several significant trends. Flower prey-derived nitrogen showed a positive correlation with soil temperature (\( p = 0.003, R = 0.493 \)). Furthermore, prey-derived nitrogen showed a significant negative correlation with total surrounding biomass (\( p = 0.005, R = -0.469 \)). It is understandable that temperature and surrounding biomass would have opposite effects on prey-derived nitrogen, as at our sample site temperature and surrounding biomass were negatively correlated (\( p < 0.0005, R = -0.478 \)). However, neither pH nor any individual plant biomass category had any significant correlation with flower prey-derived nitrogen, though Tamarac biomass was almost negatively correlated (Appendix 2).

Finally, there were several correlations found by comparing numbers of pitchers, flowers, and the weight of insects extracted from representative pitchers. A significant negative correlation was found between the number of flowers produced by a plant and the insect-derived nitrogen in the same flowers (\( p = 0.03, R = -0.372 \)). Also, when split into the categories of single flower plants and multiple flower plants, single flower plants
had a significantly higher percentage of prey-derived nitrogen in their flowers than multiple flower plants (p = 0.012), where the single flower plants averaged 79.1% prey-derived nitrogen in their flowers and multiple flower plants averaged 64.6%. Furthermore, extracted insect weight was negatively correlated with prey-derived nitrogen (p = 0.028, R = -0.376). Number of first-year pitchers showed no significant correlation with the flower prey-derived nitrogen (Appendix 2).

Differences between flowering and nonflowering plants

Flowering and nonflowering plants were separated and then means of the environmental and morphological factors were analyzed for differences. For the majority of factors, no significant difference was found between flowering and nonflowering plants. This included hair length, hair density, aperature area, pitcher length, venation, coloration, captured insect weight, pH, soil temperature, and the number of pitchers. A trend towards increased surrounding biomass near flowering plants was observed, but it was not significant (p = 0.053). Furthermore, a trend towards increased midge larvae inside flowering plants was seen when the values were normalized by taking the square root, but this also was not significant (p = 0.062). However, when separated into live and dead midge larvae, dead midge larvae showed a significant difference, where more dead larvae were found in flowering plants (p = 0.029). All other symbionts showed insignificant differences (Appendix 3).

Discussion

Prey-derived N in flowers as compared to leaves

The significantly higher amount of prey-derived nitrogen in the flowers of our samples indicates a preferential utilization of nitrogen rich in the heavier 15N isotope while building these flowering structures. There are several explanations that fit with this observation. It has been shown that Sarracenia purpurea stores prey-derived nitrogen over the winter for production of pitchers in the spring (Butler & Ellison, 2007), therefore it is likely that stored nitrogen is used for flower production as well. Therefore the difference in prey-derived nitrogen between pitchers and flowers could be explained by a preferential usage of stored insect nitrogen for flower production over pitcher production. Also, this trend can be described as a result of the same amount of prey-derived nitrogen being used to build two structures that require different amounts of total nitrogen. Since pitchers and flowers have about the same %N composition (Appendix 3), the same amount of prey-derived nitrogen would correlate to a lower δ15N in whichever structure had greater biomass. It is also possible that the elevated δ15N observed in flowers does not indicate a higher percentage of prey-derived nitrogen or that the same amount of prey nitrogen is being used in two structures of differing biomass. By using 15N to determine the source of nitrogen in the flowers, an elevated δ15N could be observed if the heavier isotope of nitrogen and not prey-derived nitrogen was selectively used in flowering structures. However, this seems unlikely, as the δ15N of plants usually reflects the signature of their nitrogen sources when nitrogen is a limiting growth factor (Evans 2001), as it is in bog ecosystems. Therefore, it is likely that flowers are elevated in prey-derived nitrogen from the pitchers of the same plant, but further experiments with nitrogen tracers would be required to rule out other options.
Factors correlating to variation in prey nitrogen

The wide variation in flower prey-derived nitrogen can be explained through correlations seen with both environmental and morphological factors. A negative correlation was observed between prey-derived nitrogen and both pitcher aperture and length. Since these two factors are indicators of pitcher size, we see a trend of larger pitchers being associated with lower prey-derived nitrogen percentages in flowers. This is likely due to trade-offs when pitchers and flowers are being produced. If a plant is producing flowers heavily with insect-derived nitrogen, there is going to be less of that nitrogen available for pitcher production, leading to smaller pitcher size. Conversely, if insect-derived nitrogen is being used to produce many large pitchers, there will be a lesser amount available for flower production.

The significant trend of plants with higher R:G ratios to produce flowers with higher prey-derived nitrogen percentages is not as easily explained. It is possible that a red pitcher attracts more insects that a similar green pitcher, but this seems unlikely. However, significant correlations were observed between R:G ratios and both soil temperature (p < 0.0005, R = 0.656) and total surrounding biomass (p < 0.0005, R = -0.619), which were both also correlated with prey-derived nitrogen in flowers (Appendix 2). Since soil temperature was positively correlated with prey-derived nitrogen, a positive correlation of R:G ratios to soil temperature would indirectly correlate increased R:G values with increased prey-derived nitrogen. Similarly, since surrounding biomass was negatively correlated with prey-derived nitrogen, increased R:G values would correlate to lower surrounding biomass and indirectly with increased prey-derived nitrogen.

The positive correlation between soil temperature and prey-derived nitrogen is likely associated with variations in microbial activity due to moss temperature. Increased soil temperature, in general, leads to increased microbial activity and therefore increased nitrogen immobilization (Binkley et al., 1994), which would also be expected to hold true in Sphagnum bogs, despite their low levels of microbial activity (Damman, 1988). Therefore, if nitrogen is immobilized and therefore unavailable to pitcher plants in areas of higher temperature, these plants are going to have to rely more on insect-derived nitrogen, which would explain increased levels in flowering structures.

Prey-derived nitrogen in flowering structures also showed a negative correlation with total surrounding biomass. In a nitrogen-limited environment, this was the opposite of what would have been expected. It was hypothesized that increased biomass would lead to increased competition for nitrogen, which would cause a pitcher plant to be more reliant on prey-derived nitrogen. However, the opposite was observed, which gives an alternate explanation. It has been shown that increased nitrogen levels will lead to increased vascular plant biomass above the bog surface (Monique et al., 2001), and that Sphagnum will have a decreased competitive advantage with increased soil nitrogen (Tomassen, 2003). Therefore, surrounding biomass is likely an indicator of increased soil nitrogen availability, which would mean that pitcher plants in these areas would also have a greater source of soil nitrogen. These plants would then not be as dependant on prey-derived nitrogen, so they would show decreased prey-derived nitrogen levels in their tissues. This agrees with the observed negative correlation between surrounding biomass and prey-derived nitrogen.

The negative correlations between the numbers of flowers with prey-derived
nitrogen are apparent in both source and significance. If a plant produces multiple flowers, it will require more nitrogen than if it produced only one flower. Since more insects cannot be caught without building more pitchers, the only source for this higher level of required nitrogen is through increased root uptake. Therefore, the prey-derived nitrogen that was used to produce flowers will be less abundant in multiple flowers than if it were concentrated all in one flowering structure. However, it is more difficult to understand the negative correlation between captured insect weight and prey-derived nitrogen. It would be expected that increased captured prey weight would lead to increased prey-derived nitrogen, but this is not the case. It is possible that remaining insect biomass relates to an inability for the pitcher to break down captured insects into a useable form, whether due to a lack of symbionts or not. This would mean that there is variation in the ability of pitchers to obtain nutrients from captured insects. More likely, it seems that current pitcher content is not an accurate reflection of the insects that are digested to produce current flowers and pitchers.

**Differences between flowering and nonflowering plants**

The difference between the number of dead midge larvae in flowering and nonflowering plants was the only significant difference in environmental and morphological factors between these two categories. This difference seems likely due to dead midge larvae becoming a nitrogen source, instead of a nitrogen sink. If a pitcher has large amounts of live midge larvae, they will be detracting from the amount of prey-derived nitrogen available to the plant. Instead, dead midge larvae are contributing to the nitrogen available to the plant. So, when midge larvae die in a pitcher, they switch from a nitrogen sink to a source, which is a contributing factor to the flowering of the pitcher plants.

**Works cited**


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