A PRELIMINARY NITROGEN COST-BENEFIT ANALYSIS OF PREY CAPTURE IN THE CARNIVOROUS PITCHER PLANT, *SARRACENIA ALATA*

by

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INTRODUCTION

Carnivory in plants has evolved independently multiple times in angiosperms (Ellison and Gotelli, 2009). These unique plants inhabit areas of high sunlight with moist, nutrient-poor soil (Givnish et al., 1984). In these environments, nutrients, particularly nitrogen (N) and/or phosphorous (P), limit growth and reproduction (Ellison, 2006). Carnivorous plants thrive in these nutrient-poor environments by capturing insect prey, digesting them, and absorbing their nutrients to supplement nutrients acquired from the soil (Schnell, 2002).

Variations in allocation of carbon and nutrients are a main focus of plant ecology (Farnsworth and Ellison, 2008; Karagatzides and Ellison, 2009). Plants allocate resources in such a way as to obtain the most limiting resource (Bloom et al., 1985). In order for carnivory in plants to be evolutionarily favored, the benefits from prey capture must outweigh the cost of the carnivorous structure (Givnish et al., 1984). Cost-benefit models are routinely employed in both ecological and evolutionary studies to examine such trade-offs (e.g., Givnish et al., 1984; Ellison and Adamec, 2011). Givnish et al. (1984) described the potential benefits of botanical carnivory and developed a cost-benefit model to explain why carnivorous plants have a competitive advantage over non-carnivorous plants in sunny, wet, environments with nutrient-poor soil. Givnish et al. (1984) posited that the primary benefit of carnivory in plants is an increased rate of photosynthesis, a hypothesis that was later supported by Farnsworth and Ellison (2008). Increased nutrient gain from prey capture increases photosynthetic rates until either light or water availability supplants nutrients as the factor limiting growth and/or reproduction

(Givnish et al 1984). Carnivorous plants are most effective in areas with high sunlight and water and low nutrient availability because they can outcompete non-carnivorous plants that lack a means of supplementing nutrient acquisition (Givnish et al., 1984). In environments that are not nutrient limited, carnivorous plants would be at a competitive disadvantage to non-carnivorous plants because carnivorous plants have very low maximum photosynthetic rates (Ellison and Gotelli, 2001; Karagatzides and Ellison, 2009). In previous studies, cost-benefit models for carnivorous plants have only been developed to analyze energetics (carbon). No study to date has examined the N costs and benefits of constructing carnivorous structures. Because N often limits carnivorous plants (Ellison, 2006), determining N costs and benefits would be beneficial when studying carnivory as an adaptive trait.

In this study, we compare the N benefit from prey capture in the pitcher plant, *Sarracenia alata,* to two different estimates of N cost. The first estimate of cost is the N content of green, mature pitchers. The second is the N remaining in pitchers after translocation (the removal of nutrients during senescence). If pitcher plants are able to translocate N from the carnivorous structure and store it for subsequent growing seasons, the amount of insect capture needed to repay the initial N investment should decrease.

MATERIALS AND METHODS

Sarracenia alata is a perennial carnivorous plant found in bogs in the southeastern United States (Schnell, 2002; Rice, 2006). The traps of *S. alata* consist of individual leaves fused along the edges to form a hollow, vertical tube (the "pitcher") (Schnell 2002; Rice, 2006). Along the abaxial side of the tube runs a thin, outward extension of the leaf known as the rib (Schnell, 2002). An oval flap of tissue, termed the hood, extends over the opening of the pitcher, but does not block the opening (Schnell, 2002; Rice 2006). Around the outer rim of the opening is a swelling, the lip or peristome, which produces nectar (Joel, 1986). Insects fall into the pitcher openings and become disoriented (Rice, 2006). Downward pointing hairs line the inside of the pitcher, preventing insects from crawling out of the pitcher (Rice, 2006). Once trapped inside the pitcher, enzymes digest the insects and the pitcher absorbs the nutrients (Schnell, 2002; Rice, 2006). Even though the pitchers occasionally capture larger insects such as Lepidoptera, Hymenoptera, and Orthoptera, the most frequently captured insects are ants (Green and Horner, 2007; Bhattarai and Horner, 2009).

It is common for pitcher plants to be damaged. Feral hogs trample the pitchers and grasshoppers chew holes in the sides of the pitchers. However, the main cause of damage in pitcher plants is larvae of moths in the genus *Exyra*. Mature *Exyra* lay eggs inside the pitcher, and the larvae feed on the inside of the pitcher leaves (Jones, 1921). Damaged pitchers were excluded from statistical analyses.

Fieldwork was conducted at a bog located near Buffalo, TX (~ 31° 18' N, 95° 59' W). Throughout the bog, twelve five-meter long transects were established. Within each transect, a young, newly opened pitcher was selected approximately every half meter, totaling 10 pitchers per transect. Selected pitchers were separated by a half meter to ensure that they were not ramets from the same genet (i.e., that each selected pitcher was a genetically distinct individual; Horner et al., in review).

Young, recently opened pitchers were exclusively selected for two reasons. First, this ensured that capture rates, which vary with pitcher age (Green and Horner, 2007; Horner et al., 2012), were similar for all pitchers in the study. Also, the newly opened pitchers had not begun capturing and digesting insects. The selected individuals were labeled with metal tags attached through the rib of the pitchers.

Selected pitchers were haphazardly assigned to one of three treatments: Benefit (B), Cost of Mature Pitchers (C) and Cost after Translocation (T). Seven pitchers in each transect were assigned to the Benefit (B) treatment. These were used to determine the N gain from insect capture. During each visit to the field, one of the seven B pitchers in each transect was randomly selected and a small cotton plug was placed inside the pitcher near the base. The cotton plug isolated previous prey and marked the start of prey capture over a designated period of time. Upon the next visit to the bog, the plugged B pitchers were measured (height and diameter), cut at the base, and collected in plastic vials. At this time, another B pitcher in each transect was randomly selected and plugged to measure capture over the subsequent time interval. This process was repeated for each of the Benefit pitchers. There were seven collection dates for B pitchers, and therefore, seven periods of capture: 10 days (June 1-10), 5 days (June 10-15), 14 days (June 15-29), 17 days (June 29-July 16), 11 days (July 16-27), 38 days (July 27-September 3), and 140 days (September 3-January 21). These collection dates correspond with 10, 15, 39, 46, 57, and 95 days after the pitchers had opened. The ideal method for measuring insect capture over time would be to remove the captured insects from the pitchers on a daily basis. This method is not possible with *S. alata* because the bases of the pitcher tubes are too narrow to recover prey without cutting the tubes open. Pitchers were collected periodically instead of at the end of the season to minimize N loss from insect prey due to digestion and absorption. If insects were collected from pitchers at the end of the season, some of their N would already have been absorbed by the pitcher, and the estimated N benefit would be an underestimate.

Two pitchers per transect were assigned to the Cost of Mature Pitchers (C) treatment. The N contents of these pitchers were used to estimate the N cost of constructing a mature pitcher. The openings of the pitchers were occluded with cotton shortly after they opened to prevent insects from being trapped and digested by the pitcher. By preventing insect capture, the amount of N in the pitcher was the same as the N originally contained in the pitcher at maturity and was not skewed by additional N absorbed from prey capture. One C pitcher per transect was measured (height and diameter) and collected 29 days after opening (June 29, 2012). The other C pitcher in each transect was measured and collected 95 days after opening (September 3, 2012).

One pitcher per transect was assigned to the Cost after Translocation (T) treatment. As with C pitchers, the opening of the T pitchers was occluded with cotton to prevent insect capture. The T pitchers were measured (height and diameter) and collected at the end of the growing season (January 21. 2013), after the pitchers had senesced. Collecting at the end of the growing season allowed the individual to translocate N from the pitcher back into the rhizomes to store for subsequent growing seasons. The N content of T pitchers represents the amount of

N that could not be translocated and used in subsequent seasons. Therefore, the N content of T plants represents the N cost of pitcher construction that cannot be recovered by translocation.

After collection, the samples were taken to a laboratory at Texas Christian University. The samples were placed in a freezer for at least 24 hours to ensure that any live prey in the pitchers was killed. The tubes of the pitchers were then opened by cutting lengthwise with a scalpel. In the C and T samples, it was confirmed that no insect capture had occurred. With the B samples, the plant material was separated from the insect capture. The insect material and plant material from all of the samples were dried to constant mass in an oven at 60^{o} C for at least three days.

The mass of the dried plant and insect samples was measured using an electronic balance. The samples were then ground into a fine powder using a Wig-l-bug™. Nitrogen and carbon contents of the samples were analyzed on a CE Elantech Flash EA CN Analyzer (Lakewood, NJ) at Texas State University (San Marcos, TX). Because some of the insect capture samples were not large enough to analyze, they were composited until a sufficient mass (4 mg) was obtained. Nitrogen content of C, B, and T pitchers was calculated as pitcher mass x proportion of N. Nitrogen content of prey capture was calculated as prey mass x proportion of N.

RESULTS

B Pitchers

To assure that average insect capture over time was not affected by changes in pitcher size between collection dates, average height and diameter of pitchers in each collection group were compared to each other. One-way ANOVAs showed that neither height [$F_{5,66}$= 0.88, p = 0.50] nor diameter [$F_{5,60}$, = 1.56, p = 0.18] varied significantly with pitcher age. The pitchers from the last collection date (235 days after opening) were not included in this analysis because the majority of them were damaged and accurate measurements could not be made. In the diameter comparisons, all pitchers from transect 11 were also removed because of damage.

Average N content of the B pitchers changed significantly over time [$F_{5,66}$ = 3.16, p = 0.013]. The N content remained relatively level until decreasing after the third collection (Figure 1).

Benefit (B)

The cumulative N capture over the entire growing season (Figure 2) was estimated by summing the N benefit of pitchers in each transect, and then taking the average of the transect totals. The cumulative N captured was 1.75mg $\pm$ 0.89 (mean $\pm$ SE) (Figure 3).

Cost of Mature Pitchers (C)

The average total N content of C pitchers collected twenty-nine days after opening (2.45mg $\pm$ 0.62) was not significantly different than that of the C pitchers collected ninety-five days after opening (2.78mg $\pm$ 0.59; $t_{0.05, 2, 22}$ = 0.389, p = 0.350) (Figure 4). The average N content across all the C pitchers was 2.61mg $\pm$ 0.42 (Figure 3).

Cost after Translocation (T)

The average N content of T pitchers was 0.002mg $\pm$ 0.0004 (Figure 3). This value represents the amount of N that the pitcher could not translocate.

DISCUSSION

Both costs of building the carnivorous structure involved approximating the N content of the pitcher. Metabolic N costs to maintain and build the pitcher were not included in this study. Because the metabolic N costs were not included, the calculated cost may be an underestimate of the true N cost to build the pitcher.

The N benefit was estimated from the insect capture by the pitcher. It is important to note that this is only an estimate. Because of the time interval between plugging the pitchers and collection of prey capture from plugged pitchers, the pitchers may have begun absorbing N from the captured insects before pitchers were collected to analyze for prey capture. Because some of the N from the captured insects may have already been absorbed by the pitcher, the amount of N in the insect capture may be an underestimate of what is available to the pitcher. Estimating benefit from the N in the bodies of captured insects may also overestimate the actual benefit because it assumes that all of the N in the insects is available to the plant. Nitrogen in the exoskeleton and other areas of the insects may not be digested and absorbed by the pitcher.

The N content of Benefit pitchers was measured in order to determine whether the N benefit from prey capture could be estimated from the N content of capturing pitchers. Despite the cumulative prey capture increasing over time, the N content in the Benefit pitchers significantly decreased [$F_{5,66}$ = 3.16, p = 0.013]. Therefore, N content of capturing pitchers is not an accurate representation of N benefit over time. The decrease in B pitcher N over time can possibly be explained by an early translocation of N in capturing pitchers.

The N content of mature pitchers (one estimate of cost) was not significantly different than the N content of prey capture (our estimate of benefit) ($t_{0.05,\,2,\,22}$ = 0.972, p = 0.171). This indicates that the N benefit derived from prey capture barely offsets the N cost of constructing a mature pitcher.

By comparing the N content of C pitchers to the N content of T pitchers, the translocation efficiency can be estimated. The average translocation efficiency among plants is 50.3% (Aerts, 1996). The N content of C pitchers was 1,305 times greater than the N content of T pitchers ($t_{0.05,\,2,\,22}$ = 4.74, P = 0.0001). The observed N translocation efficiency of *S. alata* was over 99.9%, which is a remarkable translocation efficiency that may allow *S. alata* to thrive in nutrient poor soils.

Because pitchers can translocate N to store for subsequent seasons, another N cost of building the pitcher could be estimated as the amount of N that the pitcher cannot translocate. In other words, the quantity of N in the pitcher remaining after translocation and senescence is the minimum quantity that the pitcher would have to derive from prey capture to reach a balance with N cost. The N benefit from capture was significantly greater than the N content of the T pitchers ($t_{0.05,\,1,\,22}$ = 1.972, P = 0.031), so the pitcher has more than repaid the N debt of building the carnivorous structure. In fact, prey capture resulted in a gain of 875 times more N than the post-translocation cost of construction. This large surplus was made possible by the highly efficient translocation of the pitchers.

Because of the relatively small sample size, the results should be confirmed by expanding this preliminary experiment with more individuals. The N benefit value can be made more precise by determining what percentage of the N in the insects can be absorbed by the pitcher by using labeled N.

Conclusions:

In the pitcher plant *Sarracenia alata*, the observed N benefit from prey capture barely offsets the N cost of constructing a mature pitcher. However, these plants are able to translocate over 99% of their N to store and utilize in subsequent growing seasons. The N benefit from prey capture was 875 times greater than the N cost after translocation; therefore, the pitchers not only repaid their N debt of construction, but also acquired a surplus of N.

FIGURES

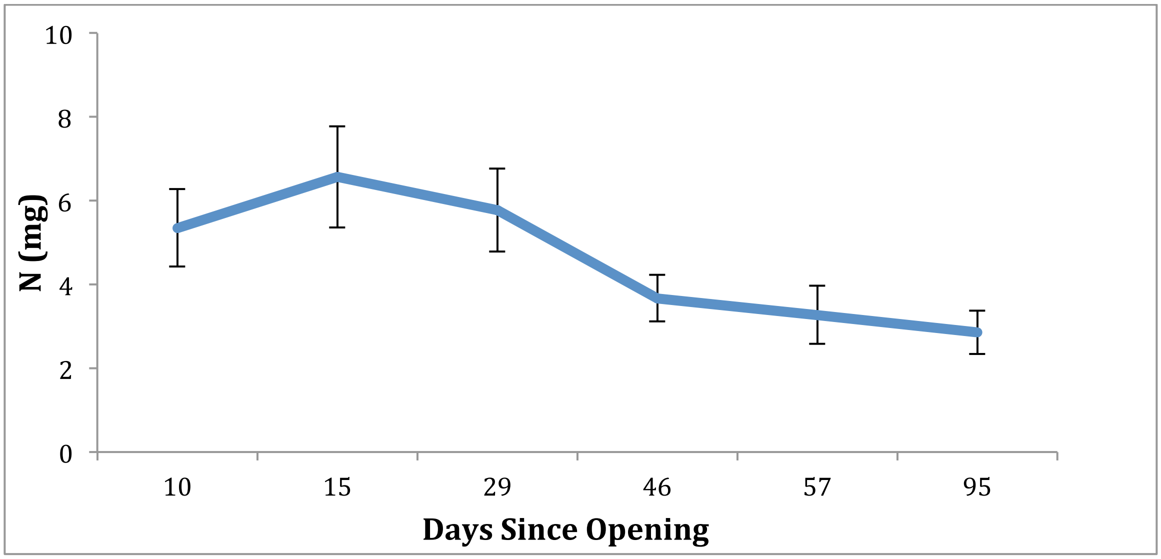

Fig. 1. Average N content of B pitchers over time

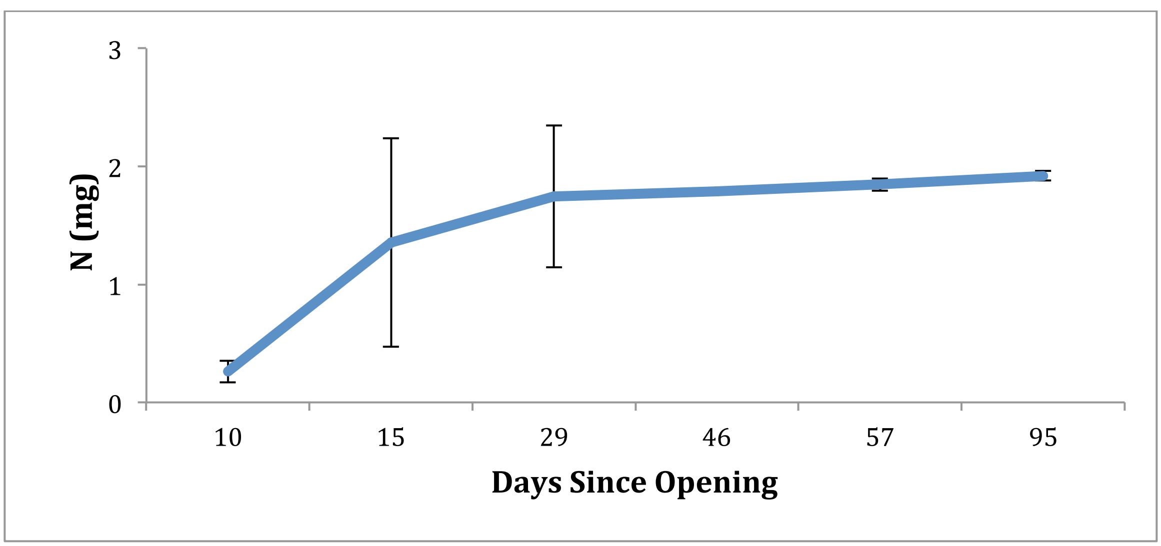

Fig. 2. Cumulative N Benefit over time

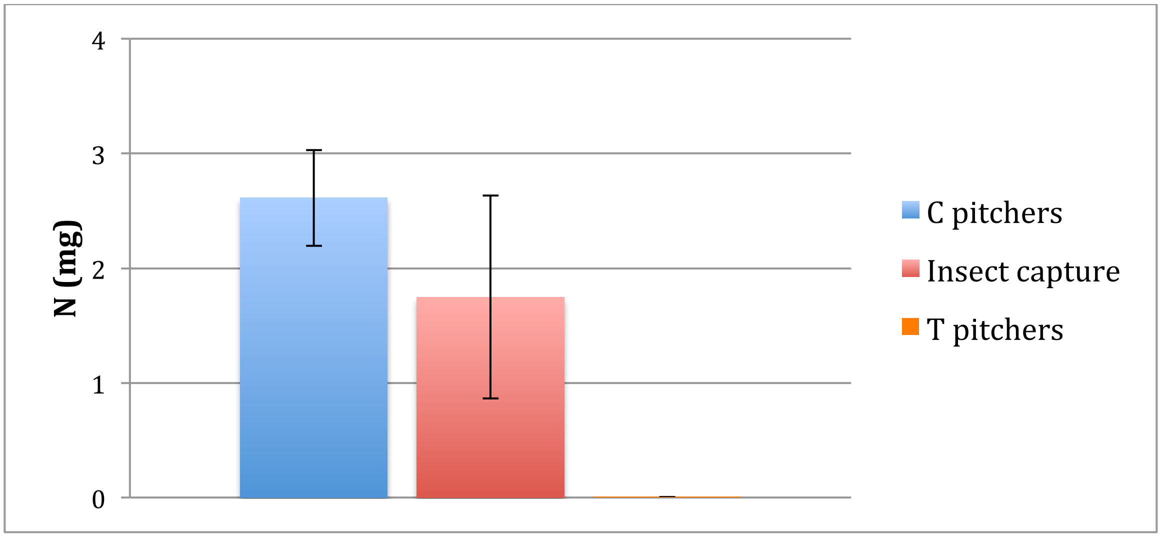

Fig. 3. Average N content of C pitchers, T pitchers, and B insect capture

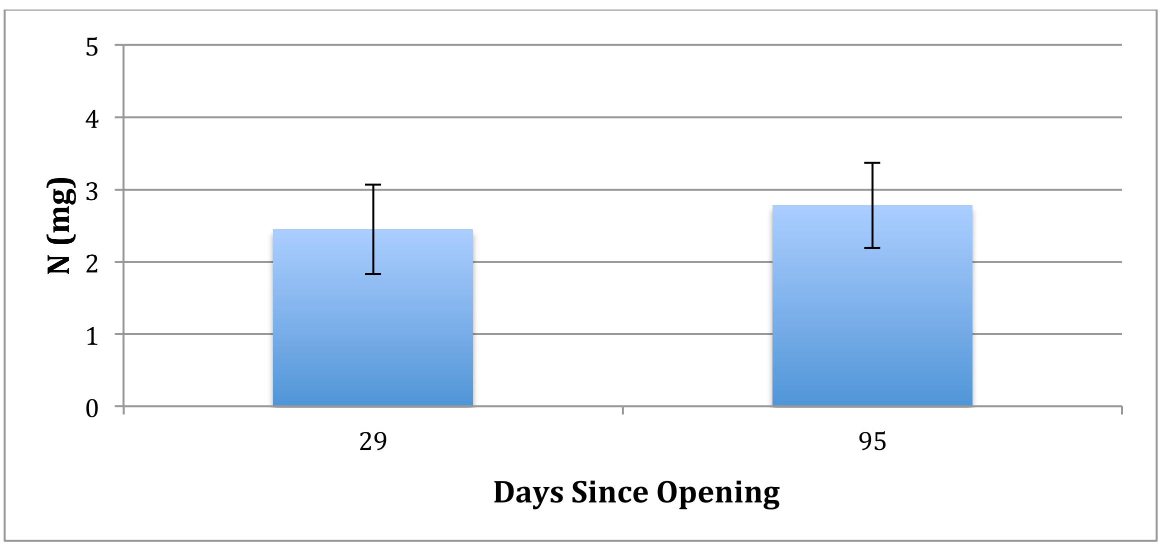

Fig. 4. Average N content of C pitchers of both collection dates

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ABSTRACT

For a trait to be evolutionarily advantageous, in theory, the benefit from the trait must outweigh the cost. *Sarracenia alata* is a carnivorous pitcher plant that grows in nitrogen-poor soil and supplements the low quantity of nitrogen (N) acquired from the soil with N absorbed from insect capture. The purpose of this study was to determine whether pitchers (the insect traps) gain sufficient N from insect capture to offset the N cost of pitcher construction. Nitrogen cost was estimated two ways, N content of mature pitchers and N content after translocation (the removal of nutrients during senescence). Benefit was estimated by measuring the total N in captured prey.

There was no significant difference between the total N content of mature pitcher tissue, which averaged 2.61mg $\pm$ 0.42 (mean $\pm$ SE), and the cumulative N in prey capture, which averaged 1.75mg $\pm$ 0.89. Therefore, the pitchers capture barely enough N to offset the N construction of a mature pitcher.

Pitcher plants can translocate N to rhizomes for use in subsequent growing seasons. Total N remaining in pitchers after translocation represents the quantity of N that cannot be recovered, and therefore the N deficit after building the pitcher. The total N in pitchers after translocation averaged 0.002mg $\pm$ 0.0004. Therefore, prey capture provided up to 875 times the amount of N that could not be recovered from the pitchers by translocation.