DECIPHERING THE IMPORTANCE OF PITCHER SIZE IN PREY CAPTURE IN THE CARNIVOROUS PLANT, *SARRACENIA ALATA* WOOD

by

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INTRODUCTION

Carnivorous plants are restricted to well-lit, moist, and nutrient-poor habitats (Givnish *et al.* 1984, Benzing 1987). Structures that trap and digest animals (usually insects) and absorb nutrients are adaptations to compensate for the nutrient-deficient soils (Schulze *et al.* 1997). Two general types of trapping mechanisms are utilized by the carnivorous plants: active traps like snap traps (*e.g.*, Venus fly traps) and passive traps like pitfall traps (*e.g.*, pitcher plants; Benzing 1987).

It has been suggested that selected characteristics of pitcher plants increase prey attraction. These characters include coloration, UV reflectance (Joel *et al.* 1985), nectar (Joel 1986, Juniper *et al.* 1989), and odor/volatiles (Miles *et al.* 1975, Juniper *et al.* 1989, Jaffe *et al.* 1995). However, no significant relationship between these characteristics and prey capture has been clearly demonstrated.

Prey capture by pitcher plants has been shown to be significantly dependent on pitcher size. For example, characteristics related to pitcher size explained 3-10 % of variation in prey capture in two studies on *Sarracenia purpurea* (Cresswell 1993, Heard 1998). Pitcher size (funnel diameter) was also the strongest determinant of prey capture in *S. alata* (Green and Horner 2007). In the latter study, differences in pitcher size explained 37-76 % of the variation in prey capture in different populations (Green and Horner 2007).

The actual reason for the importance of pitcher size in prey capture in pitcher plants is unclear. Size may be important solely because larger capture areas should allow for more prey capture. Alternatively, if there are indeed attractants produced, then larger pitcher size could also be correlated with a greater quantity of these attractants.

In this study, I examined the correlation between selected plant characteristics and prey capture in *S. alata*. I also compared the rates of insect capture by plants with those of nonbiological models and traps. The null hypothesis was that if capture rate was solely a function of capture area, then capture rates should not differ between biological and nonbiological systems of similar size (**Figure 1a**). Deviation from the null hypothesis would suggest that attractants, repellents, and/or different escape rates in plants or nonbiological models and traps have a role in prey capture (**Figure 1b**). I also examined the importance of volatiles in prey capture by comparing the effect of the extracts of pitchers and/or pitcher contents in attracting insects.

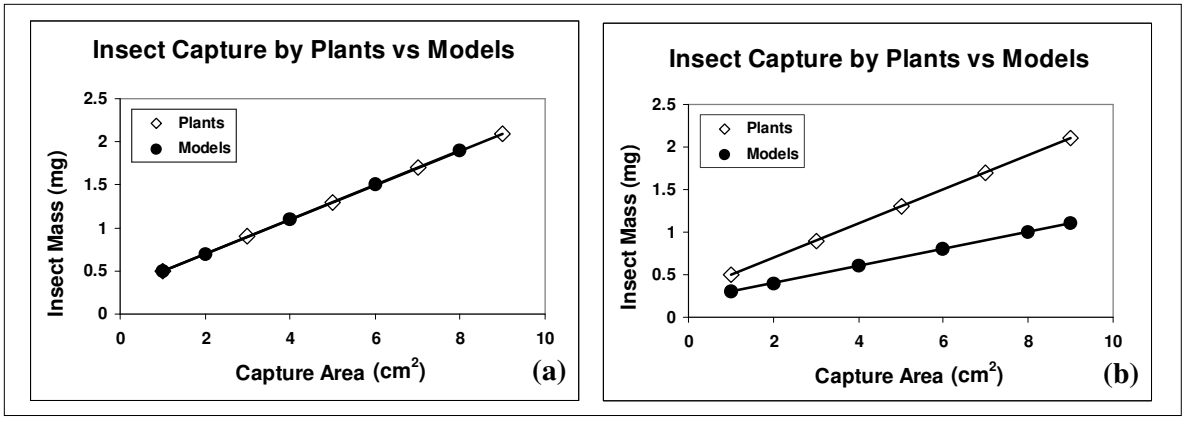

Figure 1. (a) Null hypothesis: If the capture rates of insects are similar between plants and models of similar size, it would indicate that prey capture rate is a function of area alone. **(b)** Alternative hypothesis: If the capture rate of insects is greater for plants than for models of a similar size, it would suggest that attractants, repellents, or different escape rates may play roles in prey capture.

METHODS

Study organism

Sarracenia alata Wood (**Figure 2**) is a rhizomatous, perennial herb found in bogs along the Gulf coastline from Alabama to Texas (Schnell 2002). The rhizome produces leaves in a basal rosette. The leaves develop into vertical hollow tubes called pitchers. These yellowish green pitchers are both photosynthetically active and serve as pitfall traps. The margins of the leaf meet along a vertical line (called the "rib") that extends along the length of pitcher. An oval flap called the "hood" extends over the opening but does not occlude it. The pitcher opening is surrounded by a swollen "lip." The upper part of the pitcher including the hood develops coarsely reticulated red veins. The entire outer surface of the pitcher contains numerous nectar glands that are more dense in some parts than others (Schnell 2002). The nectar, red coloration, UV reflection by the hood, and odor are believed to serve as attractants to insects (Miles *et al.* 1975, Juniper *et al.* 1989, Jaffe *et al.* 1995).

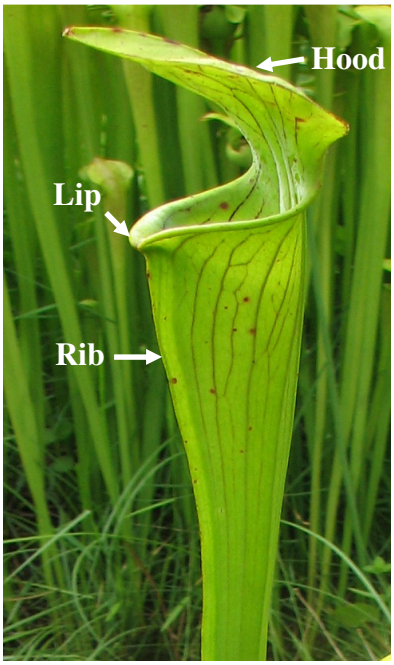

Figure 2. *Sarracenia alata.*

Correlates of prey capture

To examine the plant characteristics associated with prey capture, a field study was conducted in Hardin Bog in Leon County, Texas. Seven 6-m transects were established in the field on April 23, 2006. Newly opened pitchers closest to every 60 cm were tagged. A

small piece of cotton was pushed into the base of every tagged pitcher to mark the beginning of the study. The pitchers were allowed to trap insects for two weeks.

On May 7, 2006 the height of the pitcher (from the substrate to the lip along the rib) and the diameter of the funnel were measured to the nearest millimeter. The nectar was sampled from the lip and the outer surface (top) of the hood using separate filter paper wicks moistened with distilled water (McKenna and Thompson 1988, as modified by Green and Horner 2007). Each sample was placed in a separate screw-cap vial for storage. The hood of the pitcher was then cut in a line horizontal to the lip and placed into a snap-cap vial. Then the pitcher was cut at the base and placed together with its contents into a snap-cap vial. Upon returning to the lab, the nectar samples were oven dried at 60° C for three days. The vials containing pitchers and their contents were filled with 70% aqueous ethanol for preservation.

The contents of the pitchers were analyzed under a dissecting microscope. The contents were sorted into identifiable insects and unidentifiable mass (principally composed of partially digested insect exoskeletons, hereafter called "detritus"). Commensal insect larvae (larvae of sarcophagid flies and pitcher plant mosquitoes) and commensal arachnids (mites and spiders) were excluded from the samples. Intact insects were identified to order (using Borror and DeLong 1971). Intact insects other than ants were transferred to a new petri dish. Because of their small size, ants were left in the original petri dish with the detritus. In order to ascertain the total number of insects captured, the intact insects and all detached heads were counted. Then the insects and ants/detritus were dried at 60° C for a minimum of three days. Dry mass (to the nearest 0.1 mg) was determined for each order and the ants/detritus. A value of 0.01 mg was assigned for negligible mass. The total mass of

prey capture was determined for each pitcher as the sum of the mass of intact insects and that of the ants/detritus.

The pitcher hoods were scanned (together with a ruler for scale) into a computer at a resolution of 300 dpi. Hood area and area of red coloration were determined using the Scion Image program following the methods of Green and Horner (2007). The images were converted to grayscale and loaded into the Scion Image program. The contrast threshold for each hood was adjusted in such a way that the whole hood appeared black, and the area was measured. Then, the threshold was increased by 70 units so that only the red colored lines appeared black in the image. The area of these lines was also measured. The percentage of coloration was calculated for each hood.

Nectar was analyzed using the anthrone colorimetric technique (McKenna and Thompson 1988, as modified by Green and Horner 2007).

Forward stepwise regression was performed using SYSTAT to determine the plant characteristics related to prey capture. The independent variables were funnel area, percent of red coloration (arcsine transformed), and top and lip nectar concentrations. Separate analyses were performed for the dependent variables total mass and the mass of intact insects alone.

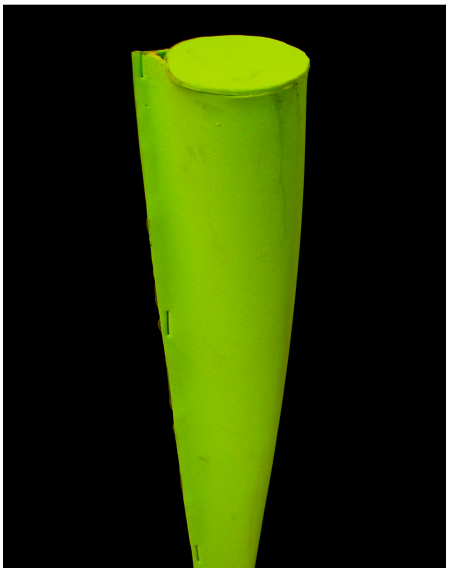

Figure 3: A model.

Insect capture by models and traps

"Models" (**Figure 3**) that simulated the shape of pitchers were constructed in the lab from 0.16-cm

closed-cell foam. They were conical, had circular tops, and were 46 cm in height. "Traps" (**Figure 4**), which were circular discs, were constructed from the same material. We used four different diameters for the traps and the tops of models: 1, 2.3, 3.6, and 5 cm. The height for models was chosen based on the average height of pitchers, and the diameters for the tops of models and the traps were chosen based on the range of funnel diameters observed by Green and Horner (2007) in other populations of *S. alata*. Gel-trap™ was applied on the upper surfaces of models and traps to capture insects.

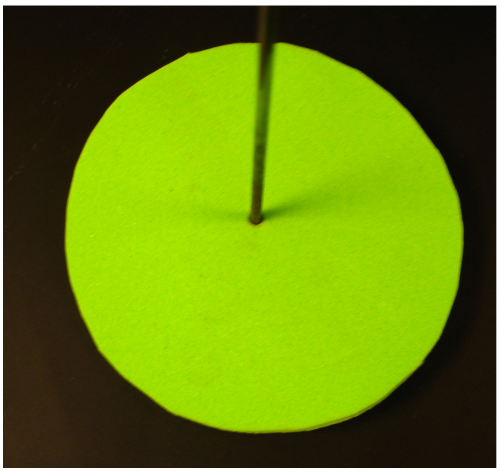

Figure 4: A trap.

Four 6-m transects were established in the field among the pitchers on the same day when transects were established for plants (see above). Two models and two traps of each size class (*i.e.,* a total of eight models and eight traps) were haphazardly assigned to positions along each transect. Traps were placed at ground level. The models and traps were allowed to capture insects for the same period of time as the plants. After two weeks, the traps and the upper surfaces of the models were covered with a thin sheet of transparent plastic and collected. Together with a ruler for scale, they were scanned into a computer at a resolution of 300 dpi. The numbers of insects captured by models and traps were counted from the image. The length of each trapped insect was determined using the Scion Image program. Insect dry mass was estimated from insect length using a regression equation generated by Rogers *et al.* (1976), which was first tested for accuracy.

Total capture was regressed as a function of area for both the models and traps. The slopes of the regression models of capture rates by plants, models, and traps were compared using analysis of covariance (Zar 1974).

To test the validity of the equation of Rogers *et al.* (1976) that was used to estimate the mass of insects captured by models and traps (and "attraction cups" later; see below), a total of 11 samples with more than 30 insects in each sample were collected by sweep netting insects on the TCU campus. Each sample of insects was transferred to a separate zip-loc bag and frozen in order to kill the insects. The bags with insects were scanned into a computer together with a ruler at a resolution of 300 dpi. Insect length was measured from the scanned images using the Scion Image program, and insect mass was estimated using the regression equation given by Rogers *et al.* (1976). The insects from each zip-loc bag were transferred to separate petri dishes and dried at 60° C for three days. Dry mass (to the nearest 0.1 mg) was determined for each sample. Expected dry mass (estimated from the equation given by Rogers *et al*. 1976) was regressed against observed dry mass. The slope of the model was compared to 1 and the intercept to 0 using Student's t-test.

Effects of volatiles on prey attraction

Experimental "attraction cups" (**Figure 5**) were constructed in the lab from plastic yogurt cups and polyethylene scintillation vials. A hole of diameter equal to that of a scintillation vial was removed from the center of the lid of the yogurt cups, and the open end of a scintillation vial was attached to the inner surface of the lid using hot glue. In this way, the cup had a scintillation vial recessed in the center of the lid. The lid was then placed on the cup, and the seam was covered with stretched parafilm.

Four 6-m transects were established in the field among the pitchers on May 11, 2007. Four different solutions were prepared: control, pitcher content, pitcher, and pitcher plus content. A distilled water (diH_2O) extract of one new pitcher was used for each sample of "pitcher," a diH_2O extract of the contents from a single pitcher was used for "pitcher content," and a diH_2O extract of a single pitcher with its contents was used for "pitcher plus content." The extract was placed into the scintillation vial in the cup and distilled water was added to bring the volume to 2/3 to 3/4 of each vial. Distilled water was used for the control. Gel-trap™ was applied on the outer surface of the lid, leaving the cavity open. Three replicates of each treatment (a total of 12 cups) were placed in each transect. One replicate of each treatment was randomly assigned to positions at 50-cm spacings along each 2-m section of the transect. The cups were left for two weeks to capture insects. On May 26, 2007, the lids of the cups were covered with a thin sheet of plastic and collected. The upper surfaces of the lids together with a ruler were scanned into a computer at a resolution of 300 dpi. The insects on each attraction cup were counted. Insect length was determined using the Scion Image program, and insect mass was calculated using the regression equation given by Rogers *et al.* (1976).

Figure 5: An attraction cup.

The total mass of insects captured by the different treatments was analyzed using two-factor analysis of variance (ANOVA). The two factors were plant and content, and each factor had two levels (present and absent). SPSS was used to perform all ANOVAs.

RESULTS

Prey capture

Insects belonging to nine orders were captured by the 63 pitchers in the study (**Table 1**). Ants were the most frequently captured group, and the ant/detritus category represented the greatest proportion of mass captured. Larger insects such as Lepidoptera, Hymenoptera and Orthoptera were captured less frequently but contributed a disproportionately greater mass per insect to total prey capture.

Table 1: Intact insect orders represented in the 63 pitchers collected

Order	Count	Proportion of counts	Mass (mg)	Proportion of mass
Hymenoptera: Ants	146	0.42	7841*	0.93
Diptera	72	0.21	127.8	0.02
Thysanoptera	62	0.18	**	**
Orthoptera	22	0.06	41.2	0.01
Hymenoptera***	16	0.05	120.7	0.02
Coleoptera	12	0.03	19.8	**
Lepidoptera	8	0.02	187.8	0.02
Hemiptera	5	0.01	**	**
Homoptera	5	0.01	**	**
Collembola	4	0.01	**	**
Total	352		8338.3	

* Including detritus mass, ** Negligible mass, *** Excluding ants

Correlates of prey capture

Funnel area was highly positively correlated with pitcher height (r = 0.69) and hood area (r = 0.86). Therefore, only one index of size, *i.e.* the funnel area, was used in the multiple regression analyses. The total mass captured by the pitchers was significantly

positively related to funnel area ($P < 0.0001$; **Figure 6).** The remaining plant characteristics (arcsine transformed area of coloration, top and lip nectar concentrations) had no significant effect on total mass of prey captured. The total mass of intact insects captured was not related to any of the pitcher characteristics.

Insect capture by models and plants

Validation of the regression equation of Rogers et al. (1976)

A total of 460 insects were analyzed in the 11 samples. The linear regression of expected mass against observed mass was significant. The slope was not significantly different from 1 ($t_{0.05, (2), 9} = 0.48$, $P > 0.5$), and the intercept was not significantly different from 0 ($t_{0.05, (2), 9} = - 0.48$, $P = 0.65$).

Insect capture by models and traps

A total of 218 insects were captured by the 26 models, and 142 insects were captured by the 23 traps. The total mass of insect capture was significantly positively dependent on capture area for both models ($P = 0.002$) and traps ($P = 3.06 \times 10^{-4}$; **Figure 6**). Analysis of covariance showed that the rates of insect capture per unit area were significantly different for plants, models and traps ($F_{0.05, 2, 106} = 556.16$). Multiple range tests revealed that the capture rate per unit area was significantly greater for plants than that for either models ($q_{0.05, 106, 2} = 28.63$; $P < 0.001$) or traps ($q_{0.05, 106, 3} = 28.11$; $P < 0.001$), and the rate of insect capture per unit area was slightly but significantly greater for models than for traps ($q_{0.05, 106, 2} = 4.04$; $P < 0.025$).

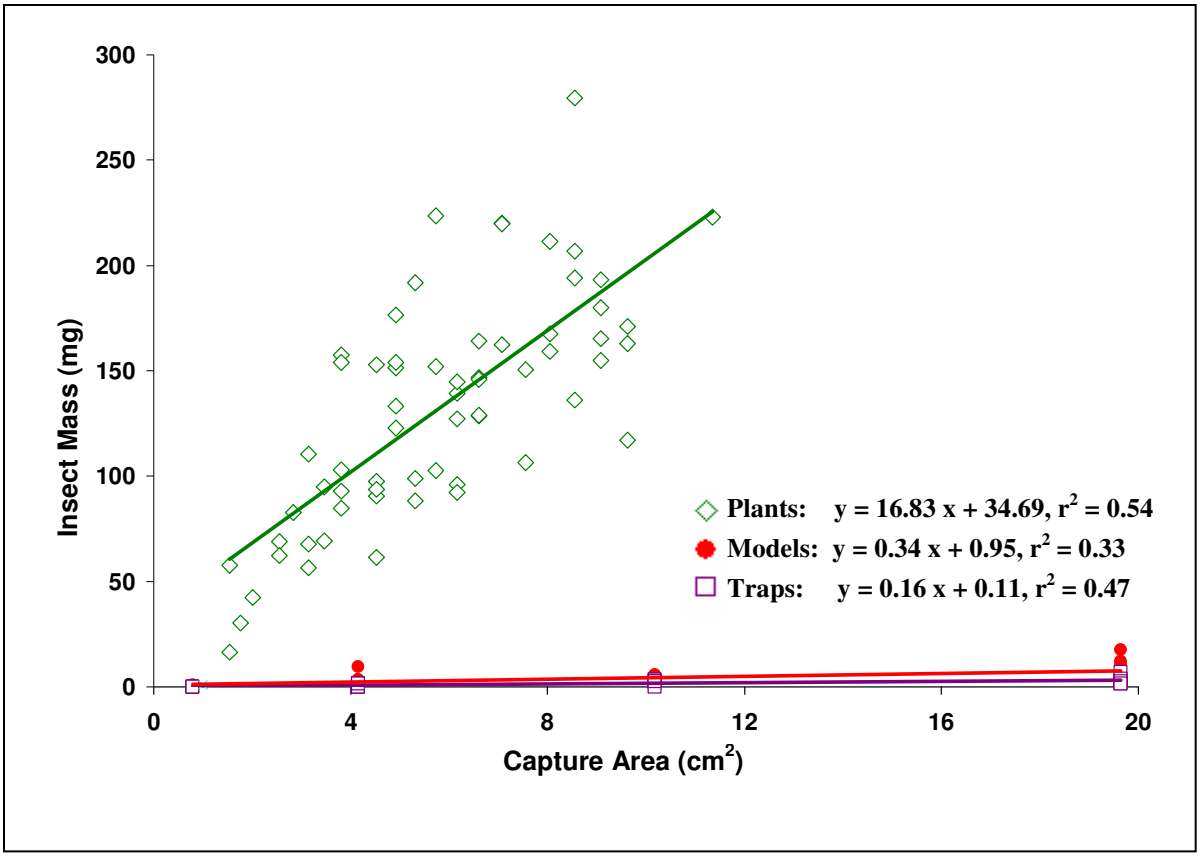

Figure 6: Insect capture as a function of capture area for pitchers, models, and traps.

Effect of volatiles on prey capture on attraction cups

A total of 1267 insects were captured by the 45 attraction cups analyzed. Treatment significantly affected the log mass (mg) of insects captured ($F_{3, 41} = 4.66$, $P < 0.01$), and there was a significant interaction between pitcher and pitcher content. Attraction cups containing pitcher contents captured significantly greater (log) mass of insects than the control, but the insect mass captured by the former was not significantly different from that of attraction cups containing an extract of either pitcher or pitcher plus content. The mass of insects on attraction cups containing extracts of either pitcher or pitcher plus content was not significantly different than the control (**Figure 7**).

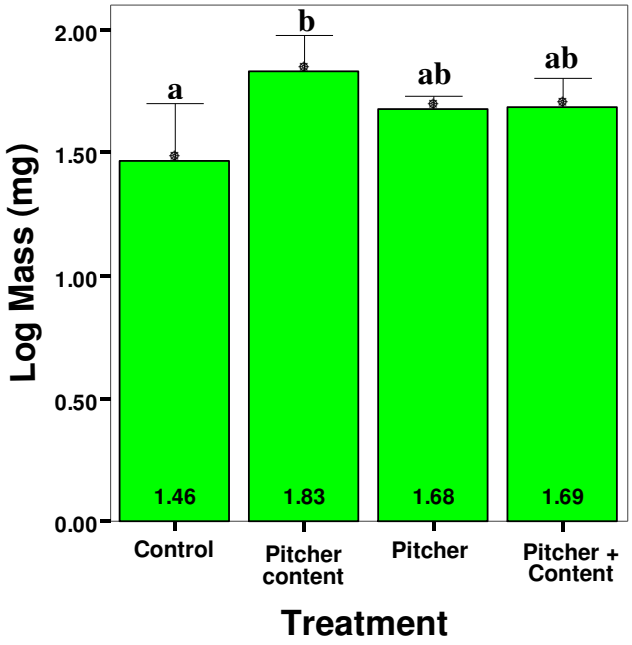

Figure 7: Effect of volatile treatments on insect capture by attraction cups. The bars represent means of the log mass (mg) of insect capture, and error bars show 95% confidence interval of means. Bars with the same letters are not significantly different.

DISCUSSION

Funnel diameter was the only plant characteristic measured that was significantly related to the total mass of insects captured. The rate of insect capture per unit capture area of plants was significantly greater than that for models and traps. This result could be explained in different ways, such as a higher rate of insect escape from the models and traps than from plants, the presence of repellents in the models and traps, and/or the presence of attractants in plants. A very high rate of insect escape has been reported in a related species of pitcher plant, *S. purpurea* where less than 1% of potential prey were captured (Newell and Nastase 1998), while almost no insect visiting a gel-trap coated model or trap can escape

(personal observation). Gel-trap is widely used by ecologists to capture insects, so the presence of repellents in gel-trap is unlikely. The 0.16-cm closed-cell foam was green and odorless, and had no discernible effect on movement of crawling insects. Therefore, the presence of attractants in the plants is the most plausible explanation for the higher rate of insect capture by plants than by models and traps of similar size.

The significantly greater insect capture by the attraction cups with the extract of pitcher content compared to control cups may indicate the importance of the odor of decaying insects in attracting insects. The effect of the odor of decaying insects present in the combination treatment might be diluted by the presence of plant volatiles. The odor of decaying insects may be one of the most important volatile attractants, but it doesn't appear to explain prey capture by newly opened pitchers. This might indicate the role of other attractants such as nectar and/or UV reflectance.

Although we found no relationship between nectar concentration and prey capture, this may be because of our methodology. Pitchers produce nectar over the entire outer surface and throughout their lifespan, though the nectar concentration differs among the pitcher parts and with age (Horner, unpublished data). It might be that insects are attracted toward a pitcher because of the total amount of nectar produced over the entire surface of the pitcher rather than its concentration at a particular region. Likewise, the total mass of prey captured by pitchers over a period of time may be a function of total nectar produced over that time period. Therefore, our measurement of nectar concentration with no estimate of total production may have prevented us from observing a relationship between nectar and prey capture.

The majority of insects captured by the attraction cups were flying insects. Crawling insects (including ants, which constitute a major proportion of prey capture in pitcher plants) might be lured by attractants other than volatiles. Different insects might be lured by different attractants, and the change in attractants with pitcher age (Horner, unpublished data) might result in variation in the type of prey captured over time.

Models had a slightly but significantly higher rate of insect capture per unit capture area than the traps. This might be the result of difference in heights of models and traps. Both models and traps captured similar insects, the majority of which were flying insects. Therefore, models may have captured more insects simply because they were taller.

This study demonstrates that the effect of size on prey capture in pitcher plants is not due to differences in capture area alone. Rather, our results are consistent with the presence of attractants. Larger pitcher size could equate to a greater quantity of attractants which ultimately results in a higher rate of prey capture. Therefore, prey capture by plants is not simply a function of capture area, but it may also reflect differences in the quantity of attractants. Although we found that volatiles have some effect on prey attraction, the precise nature of all of the attractants is yet to be determined.

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ABSTRACT

DECIPHERING THE IMPORTANCE OF PITCHER SIZE IN PREY CAPTURE IN THE CARNIVOROUS PLANT, *SARRACENIA ALATA* WOOD

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Prey capture in pitcher plants has been found to be significantly dependent on pitcher size, but the actual importance of size is not clearly understood. We studied insect capture by the carnivorous plant *Sarracenia alata* and compared the rate of insect capture per unit capture area of plants with that of nonbiological models and traps. The total mass of insects captured was significantly positively related to capture area for both biological and nonbiological systems, explaining 54% of variation in plants, 33% in models, and 47% in traps. The rate of insect capture was significantly greater for plants than for models and traps, which suggests the role of attractants in insect capture in pitcher plants. Odor from decaying insects was found to have a significant effect on insect capture in attraction cups. Further study should focus on the nature of other attractants including nectar, UV reflectance, and volatiles to decipher the mechanism of insect capture by pitcher plants.