

Potential Effects of Elevated CO₂ on Pitcher Plant Nectar Composition, Prey Capture, and Inquiline Communities

Abstract

Human activities such as fossil fuel burning and deforestation have contributed significantly to the increasing levels of CO₂ in the atmosphere since the industrial revolution. Under A1FI emission scenario we are expected to reach rates as high as 1000 ppm by end of century. Studies have shown that C₃ type plants experience a 27% increase in C:N ratios under elevated CO₂. Many of these plants will experience a boost in plant growth. Studies have shown that these plants will increase in carbon-based compounds such as sugar in the nectar of plants and decrease in nitrogen. Carnivorous plants are a rare type of C₃ plant that have adapted to survive in limiting nutrient soils. For the insectivorous pitcher plant *Sarracenia purpurea*, is a special kind of C₃ plant that provides an aquatic habitat for mosquito larvae and midge larvae and captures its prey by lure to obtain certain nutrients. This study focuses on the potential effects of elevated CO₂ on pitcher plant nectar composition, prey capture, and inquiline communities. We hypothesize that capture rates and inquiline abundance will be strongly related to the amount of nectar present on the lip of the pitcher. Further we predict that due to increased C:N and future elevations in CO₂ will have positive effects on pitcher plants and the insect communities they support.

Introduction

According to the U.S. National Oceanic and Atmospheric Administration (NOAA), pre-industrial carbon dioxide (CO₂) levels were fairly stable at approximately 280 ppm until 1850. Human activities such as burning fossil fuels and deforestation have increased those levels up to 380 ppm by early 2006. The atmosphere now has about 400 ppm CO₂, which is higher than any time in recorded history. This number is rising by about 2 parts per million every year. Current projections are for concentrations to continue to rise to as much as 500–1000 ppm by the year 2100 (IPCC 2007).

For most plants, growth under elevated CO₂ results in increased biomass since photosynthetic rates are increased; however, this increase is accompanied by a decrease in leaf percent nitrogen and an increase in C:N (Kelly et al., 2013). A study by Cotrufo et al (1998) found that elevated CO₂ reduces nitrogen concentration of plant tissues. The study found that elevated CO₂ appeared to have different effects on the N concentrations of different plant types more reduction in C₃ plants over C₄ plants and N₂-fixers (Cotrufo et al 1998). Therefore, in the future, plants may have less nitrogen available for important internal processes (Long et al., 2004). Stiling and Cornelissen found that C₃ plants will also experience a 27% increase in C:N

under elevated CO₂ (2007). Higher carbon-to-nitrogen ratios results in increased carbon based compounds such as carbohydrates and secondary compounds not involved in primary metabolism.

Carnivorous plants are plants that derive some or most of their nutrients from trapping and consuming animals or insects. Carnivorous plants have adapted to grow in places where soils are a very poor source of nutrients, especially nitrogen. Carnivorous plants typically grow in acidic, boggy areas where plant food and nitrogen sources are considerably low. There are over 670 species of carnivorous plants around the world. These plants are fascinating to most humans for their abilities to attract and digest insects. There are four major types of carnivorous plants and they are the fly traps, snap traps, bladder traps and pitfall (pitcher) plants.

Pitcher plants are one of the major types of insectivorous plants. Pitcher plant leaves wrap into “pitcher” or vase like shape. Pitcher plants can grow to heights of 4-42 inches tall and widths of up to three feet (bhg.com/gardening/plant-dictionary). Some pitcher plants are green and others are darker red in color depending on the level of venation. Pitcher plants are considered to be less active and passive in nature as they do not have movement. Insects are instead attracted by sweet nectar and color of pitcher plants. Some studies have found that color was a major attractant while others show nectar as primary attractant. All 110 species of pitcher plants possess extra-floral nectaries (EFN) (Juniper et al. 1989; Vogel 1998, Bennett et al. 2009). Nectar production increases prey capture rates by decreasing friction on pitcher lips (Bauer et al. 2008, Bennett et. al 2009), and thereby increasing the probability that prey fall into the pitcher. Once inside the pitcher plant the slippery EFN along with sharp downward pointing hairs makes it difficult for insects to escape. The insect falls to the bottom of the pitcher plant where it drowns in standing water (microscopy-uk.org/uk/mag/artnov06macro/hm-macro.html).

Studies have found that EFN produced by nectaries on leaves, petioles, stipules and stems, contain both carbon based sugars and nitrogen based amino acids. (Baker et al., 1978; Dress et al. 1997; Deppe, 2000). In another study by Cipollini et al found that carbohydrates are found in the nectar located on and around lip (mouth) of the pitcher (1994). Amino acids occur in extra-floral nectars and differ in concentration, influence insect behavior and also attract ants, wasp and other insects that serve to protect the plant from herbivory (Dress et al., 1997). Extra-

floral nectar produced by the pitcher plant *Sarracenia purpurea*, attracts insects not for protection of herbivores but as prey for their water-filled trap (Dress et. al, 1997).

Study Species

Sarracenia purpurea, the northern purple pitcher plant (Fig. 1), is commonly found in North America, Canada and small portions of Florida. *Sarracenia purpurea* is commonly found and adapt well in cold regions. It is generally believed that *Sarracenia purpurea* lures its prey with a variety of traits including color and nectar (Fig. 3). However, some studies have not found evidence that nectar attracts prey. For instance, Green et al. (2007) and Creswell (1993) found no correlation between sugar concentration of nectar and prey biomass captured by individual pitchers (Creswell 1993). In some studies, pitcher size was positively correlated with prey capture (Creswell 1993). Other studies have shown that color attracts prey with more red color (Nastase & Newell 1998). In contrast, other studies have indicated that nectar (Fig. 2) may be a more important attractant than color (Bennett & Ellison 2009; Newell & Nastase 1998). For instance, using artificial pitchers, Bennett and Ellison (2009) found that the number of ants captured was unrelated to the proportion of color (red) but positively correlated with the presence of nectar. *Sarracenia purpurea*, is also home to inquiline communities and each water-filled pitcher hosts a small aquatic invertebrates (Harvey & Miller 1996). In this system exist local communities of bacteria, mites, rotifers, protozoans and mosquito larvae (Metacommunities, pg. 184).



Fig. 1 green and red pitcher plants.



Fig. 2 nectar of pitcher plant



Fig. 3 fluid with prey capture

Since elevated levels of CO₂ cause an increase in C:N ratio in most plants, and likely in pitcher plants, future elevated levels of CO₂ may also cause an increase in the amount of sugar. If so, the continued burning of fossil fuels may alter the ability of pitcher plants to capture prey. Therefore, in this study I ask:

1. Do carbon-based sugars in nectar affect the total biomass of prey captured and/or the specific types captured by a pitcher?
2. Do sugars in nectar affect the number of inquilines and/or the specific types of inquilines within a pitcher?
3. In today's plants, is C:N correlated with sugar content of nectar? If so, how will higher C:N under future elevated CO₂ affect nectar sugar content, prey capture, and inquiline communities?

Materials and Methods:

Field Site:

This study was conducted in summer of 2013, at Mud Lake Bog, in Cheboygan County, Michigan.

Sampling

Our initial visit on July 1, 2013 we randomly identified and selected 8 pitcher plants for sampling. Pitcher colors ranged from green to dark red (with some variation in between). Nectar samples were obtained from the lips of each pitcher plant (avg. length 2.64 cm) by applying one dry wick to left side of pitcher lip with vinyl paperclip and let stand for 30 min. On the right hand side of the lip a wet wick was held in place for 10 seconds to absorb nectar. After appropriate time each wick was removed with or without forceps and place into individual microcentrifuge tubes. Each tube was labeled [1a (dry) – 1b (wet), 2a (dry) – 2b (wet) . . .] taken back to the lab and frozen at -20⁰ C and held until analysis.

1st Sugar Analysis

Wicks were measured for total quantity of sugar collected from paper wicks. We determined sugar content by colorimetric anthrone assay modified by microliter volumes (Cipollini et al (1994); Deppe et al (2000)). Sugars from dry (a) and wet (b) wicks were redissolved by vortexing for one minute in 3 ml MilliQ water; separate standards were created for each dry and wet sample. In screw-cap glass test tubes we combined 2 ml of sugar standards with 4 ml of anthrone reagent (0.4 g in 200 ml concentrated sulfuric acid). Reagent blanks and sugar standards (dry/wet wicks) were vortex for 10 seconds and allowed to cool at room

temperature. Read ABS at 620 nm, using a spectrophotometer (Cipollini et al (1994) and recorded each measurement of sugar. This method was used to determine and quantify sugar on the lip of pitcher plants.

After determining that nectar was present on the lips of the pitcher plants and that sugar was present within the nectar we identified and sampled 25 pitchers plants that would be used to conduct this study. On three separate occasions (July 16, 2013, July 21, 2013 & July 28, 2013) each pitcher plant was bagged with bridal veil for 24 hour period before collection. Nectar was collected from each pitcher plant by wet paper wicks, labeled and placed in microcentrifuge tubes and taken to the lab, held and frozen at -20° C and held until analysis.

On *July 16, 2013* each plant was sampled for nectar by wiping with wet filter wicks on inside lip, outside lip and across top of lip and recording times after each collection. Thermocouple was used to measure temperatures of each pitcher plant. Dowels were put in place and each pitcher was covered with bridal veil and held in place by twist tie. On July 17, 2013 each bridal veil lifted and temperature was taken after 24hr period elapsed and removed. After removal of bridal veil another nectar sample was taken, bridal veils were collected

On *July 21, 2013* each plant was sampled for nectar by wiping with wet filter wicks on the inside of lip and across the top of lip and time was taken after each collection. Thermocouple was used to measure temperature of each pitcher plant. Dowels were put in place and each pitcher was covered with bridal veil. On July 22, 2013 each bridal veil was lifted and temperature was re-taken after 24hr period and placed in labeled microcentrifuge tube. After collecting nectar samples each pitcher lip was measured.

On *July 29, 2013* each plant was sampled for nectar by wiping with wet filter wick on inside of lip, outside lip and across the top of lip and time was taken after each collection. Thermocouple was used to measure temperatures of each pitcher plant. Dowels were put in place and each pitcher was covered with bridal veil and held in place by twist tie. On July 30, 2013 each bridal veil was lifted and temperature was taken after 24hr period and placed in labeled microcentrifuge tube. After nectar collection on this day each pitcher plant was measured for (mouth) size of opening. Studies have shown that both insect biomass is significantly positively related to biological and nonbiological systems including pitcher plant size (Bhattarai & Horner, 2008).

2nd set of Sugar Analyses (July 20, 2013), (July 24, 2013) & (July 30, 2013)

Wicks were measured for total quantity of sugar collected from paper wicks. We determined sugar content by colorimetric anthrone assay modified by microliter volumes (Cipollini et al (1994); Deppe et al (2000)). Sugars from wet wicks were redissolved by vortexing for one minute in (see chart) MilliQ water; separate standards were created for each sample. In screw-cap glass test tubes we combined sugar standards with 4 ml of anthrone reagent (0.4 g in 200 ml concentrated sulfuric acid). Reagent blanks and sugar standards were vortex for 10 seconds and allowed to cool at room temperature. Read ABS at 620 nm, using a spectrophotometer (Cipollini et al (1994) and recorded each measurement of sugar. This method again was used to determine and quantify sugar on the lip of pitcher plants.

#	ABS July 20	Extract (ml)	ABS20/ml	Temp20	ABS July 24	Extract (ml)	ABS24/ml	Temp24	ABS3	Extract (ml)	ABS3/ml
1	0.220	0.3	0.733	38.5	0.130	1.0	0.130	19.2	0.133	0.5	0.266
2	0.075	0.3	0.250	36.3	0.068	1.0	0.068	22.7	0.582	0.5	1.164
3	0.560	1.0	0.560	36.0	0.065	1.0	0.065	17.7	0.073	0.5	0.146
4	0.184	0.3	0.613	39.0	0.051	1.0	0.051	17.6	0.090	0.5	0.180
5	0.084	0.3	0.280	38.3	0.463	1.0	0.463	17.7	0.363	0.5	0.726
9	0.376	1.0	0.376	34.0	0.197	1.0	0.197	21.9	0.354	0.5	0.708
11	0.425	1.0	0.425	32.7	0.263	0.5	0.526	27.9	0.265	0.5	0.530
12	0.378	1.0	0.378	35.7	0.081	1.0	0.081	24.2	0.076	0.5	0.152
13	0.146	1.0	0.146	41.7	0.292	1.0	0.292	24.1	0.088	0.5	0.176
14	0.121	1.0	0.121	36.1	0.238	1.0	0.238	25.6	0.159	0.5	0.318
16	0.154	1.0	0.154	36.6	0.045	1.0	0.045	23.4	0.257	0.5	0.514
17	0.543	1.0	0.543	40.5	0.572	1.0	0.572	25.8	0.023	0.5	0.046
18	0.162	0.3	0.540	35.0	0.304	1.0	0.304	25.8	0.310	0.5	0.620
19	0.142	0.3	0.473	36.3	0.298	1.0	0.298	19.3	0.278	0.5	0.556
20	0.118	1.0	0.118	36.3	0.268	1.0	0.268	20.9	0.243	0.5	0.486
21	0.313	1.0	0.313	36.7	0.069	1.0	0.069	21.8	0.026	0.5	0.052
22	0.095	0.3	0.317	37.2	0.524	1.0	0.524	24.3	0.106	0.5	0.212
23	0.000	1.0	0.000	37.9	0.277	0.5	0.554	23.2	0.230	0.5	0.460
24	0.124	0.3	0.413	35.0	0.465	1.0	0.465	23.8	0.216	0.5	0.432
25	0.164	0.3	0.547	37.9	0.281	0.5	0.562	24.6	0.098	0.5	0.196
26	0.525	1.0	0.525	34.9	0.166	1.0	0.166	23.6	0.257	0.5	0.514
27	0.210	0.3	0.700	36.2	0.267	1.0	0.267	24.2	0.033	0.5	0.066
28	0.201	1.0	0.201	38.7	0.079	1.0	0.079	24.3	0.224	0.5	0.448
29	0.416	1.0	0.416	37.6	0.093	1.0	0.093	26.4	0.435	0.5	0.870

Table 1 – Pitcher plant number, sugar standard extraction amounts, temperature and absorbance

Prey collection and identification

On July 31, 2013 the fluid (rain water) and insects were removed from pitcher plants with turkey baster and syringe injection. A way to insure all insects were collected was to use 10cc of de-ionized water into each pitcher. Inquilines were identified (see Table 2) and separated using fine forceps and placed collectively in a petri dish. Using a dissecting microscope each prey head capsule was counted and identified (see Table 2). Prey sample containers were placed in drying oven at $\sim 70^{\circ}$ F and allowed to dry for about 2 days then weighed using a balance. Weights for overall biomass were recorded for each pitcher plant for statistical reports.

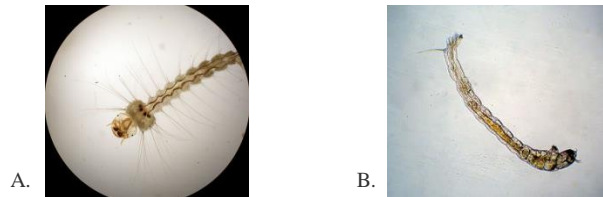


Figure 3. Images of inquilines A) Mosquito larva, B) Midge larva

Table 2. Total prey individuals and inquilines

Pitcher #	Inquilines			Prey		
	Mosquito larvae	Midge larvae	Fly larvae	Ants	Mosquitos	Total Number
1	34	0	0	0	1	35
2	29	1	0	9	2	41
3	20	6	0	0	1	27
4	15	3	0	0	0	18
5	52	6	1	0	12	71
9	11	1	4	0	0	16
11	39	4	2	1	1	47
12	3	0	0	0	0	3
13	0	0	0	0	0	0
14	18	4	0	0	0	22
16	6	2	0	0	0	8
17	3		0	0	0	3
18	0	2	0	0	0	2
19	0	16	0	0	2	18
20	19	0	3	0	0	22
21	1	0	2	0	1	4
22	2	0	0	1	0	3
23	16	0	0	0	0	16
24	30	6	0	2	2	40

25	16	18	0	2	0	36
26	19	0	0	1	0	20
27	16	1	1	2	1	21
28	10	1	0	1	0	12
29	9	0	0	0	0	9

C:N & Color determination

On *August 3, 2013*, lips were removed from pitcher plant for the purpose of C:N and photos were taken of each pitcher plant. Lips were removed and placed in small glassine envelopes then placed in large Ziploc bag, returned to lab and placed in freezer at -20° C until further analysis. The glassine envelopes were removed from freezer and Ziploc bag on August 4, 2013 and placed in Lyophilizer for freeze-drying. Once the pitcher plant lips were removed from the Lyophilizer they were ground by using pestle and mortar and liquid nitrogen. The ground lip place in microcentrifuge tubes, placed in drying oven for ~ 15 minutes, weighed and submitted for C:N analysis.

The hoods of each pitcher plant were photographed to determine color variation between pitcher plants. Adobe Photoshop was used to quantify (25%) redness of each pitcher plant. The quick selection dropper was used to select entire section of hood and lip area then total number of pixels was recorded. The “red” color range was selected within the 25th percentile and calculated by dividing the range and standard deviation in percent red by the mean percent red. Redness was tested to look at other confounding variables that may influence the increase of insects capture and visits.

Results and Discussion

Pitcher plants that produce more sugar captured more prey overall indicated by significant correlations between total prey biomass and sugar in nectar ($R^2 = 0.18$, $p = 0.33$). The R^2 value is not very high and sugar only explains about 18% of the variation of prey biomass however, statistically significant (Fig. 4).

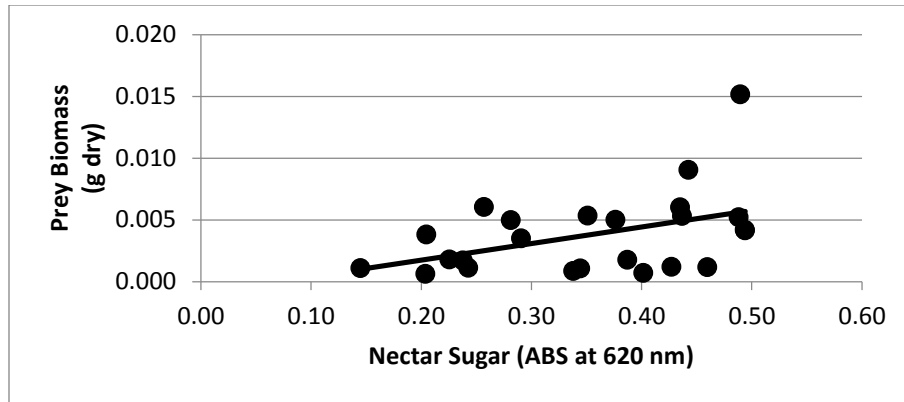


Figure 4. Relationship between sugar in nectar (x-axis) and prey biomass (y-axis) of 24 pitcher plants.

Pitcher plants that produced more sugar capture more total prey individuals overall ($R^2 = 0.18$, $p = 0.38$) (Fig. 5a), appear to be particularly more attractive to ants ($R^2 = 0.18$, $p = 0.034$) (Fig. 5b) as indicated by significant relationships and nearly significantly attract more mosquitos ($R^2 = 0.12$, $p = 0.092$) (Fig. 5c).

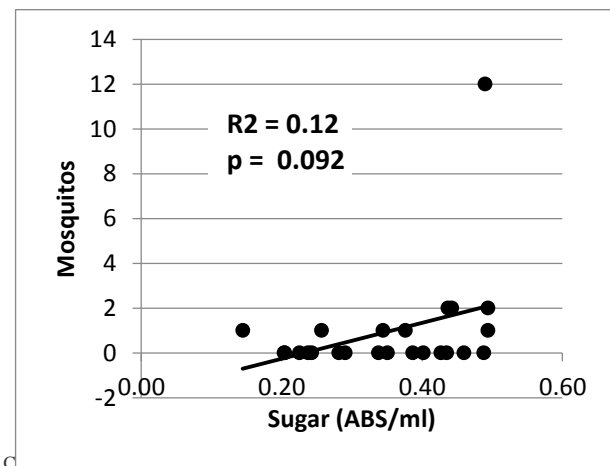
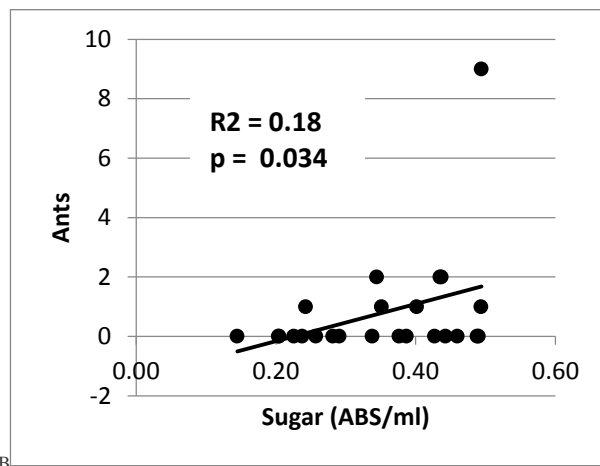
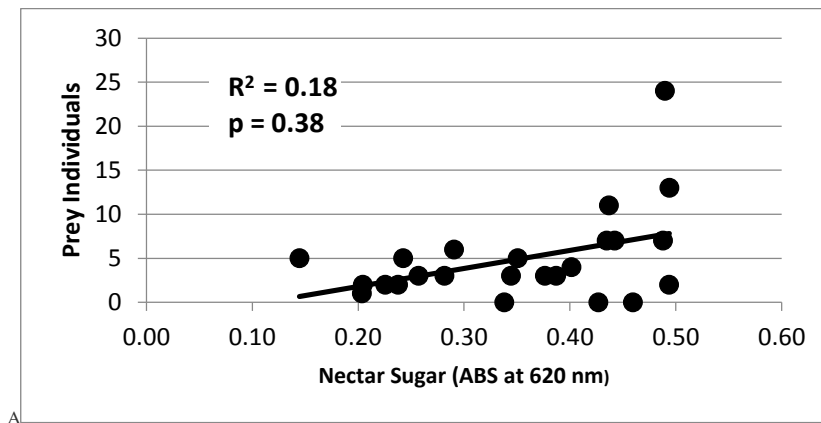


Figure 5. Relationship between sugar in nectar (x-axis) and (A) Prey Individuals (y-axis), (B) Ants (y-axis), (C) Mosquitos of 24 pitcher plants.

Pitcher plants that produce more sugar have more total inquilines including mosquito larvae (Fig. 9). Pitcher plants with sugar that attract more prey may allow ovipositing female mosquitos to choose better pitchers for their offspring.

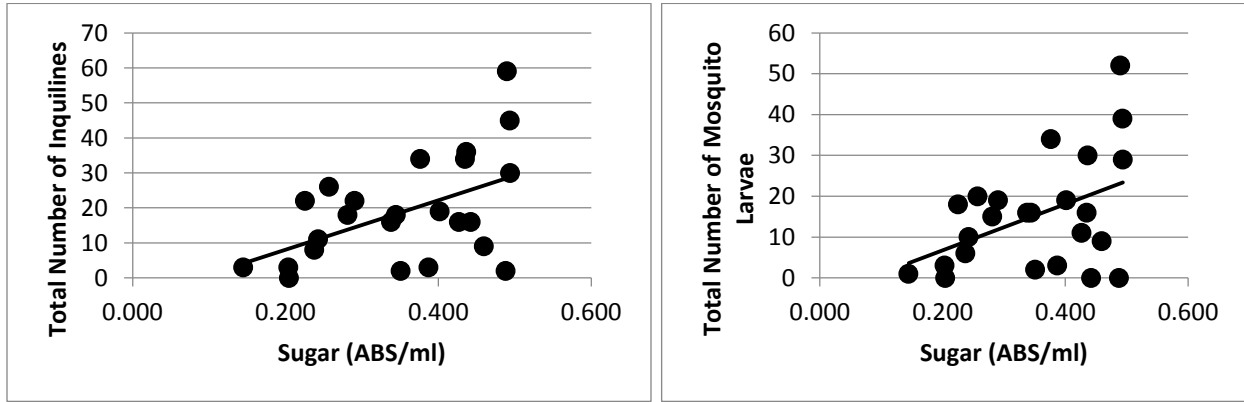


Figure 9. Relationship between sugar in nectar (x-axis) and (A) Total number of inquilines (y-axis), (B) Mosquitos (y-axis) of 24 pitcher plants.

Pitcher plants with higher C:N ratio were found to produce nearly significantly more sugar ($R^2 = 0.14$, $p = 0.067$) overall indicating a positive and nearly significant relationship (Fig. 10).

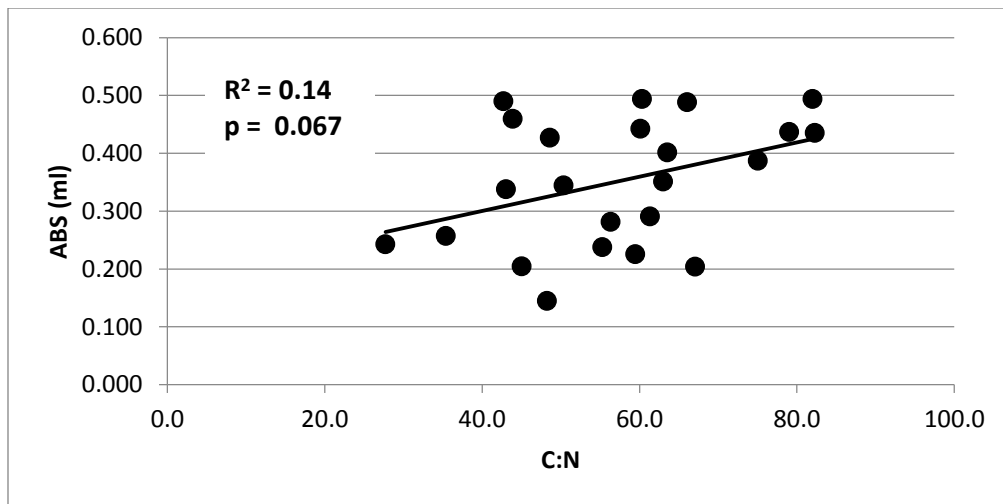


Figure 10. Relationship between C:N (x-axis) and sugar in nectar of 24 pitcher plants.

Results of this study show a significant correlation between pitcher plants that produce more sugar and insect prey capture rates. We also found a significant relationship in the amount of pitchers that produce more sugar and abundance of inquilines. Pitchers with higher C:N produce nearly significantly more sugar. Therefore, as atmospheric CO₂ increases and pitcher C:N increases, pitcher plants are likely to produce more sugar and, as a consequence, capture more prey and support a more abundant inquiline community. We provide preliminary calculations as a way to infer future prey capture rates: A 27% increase in C:N and nectar sugar will result in pitchers capturing an additional 1.2 milligrams of prey.

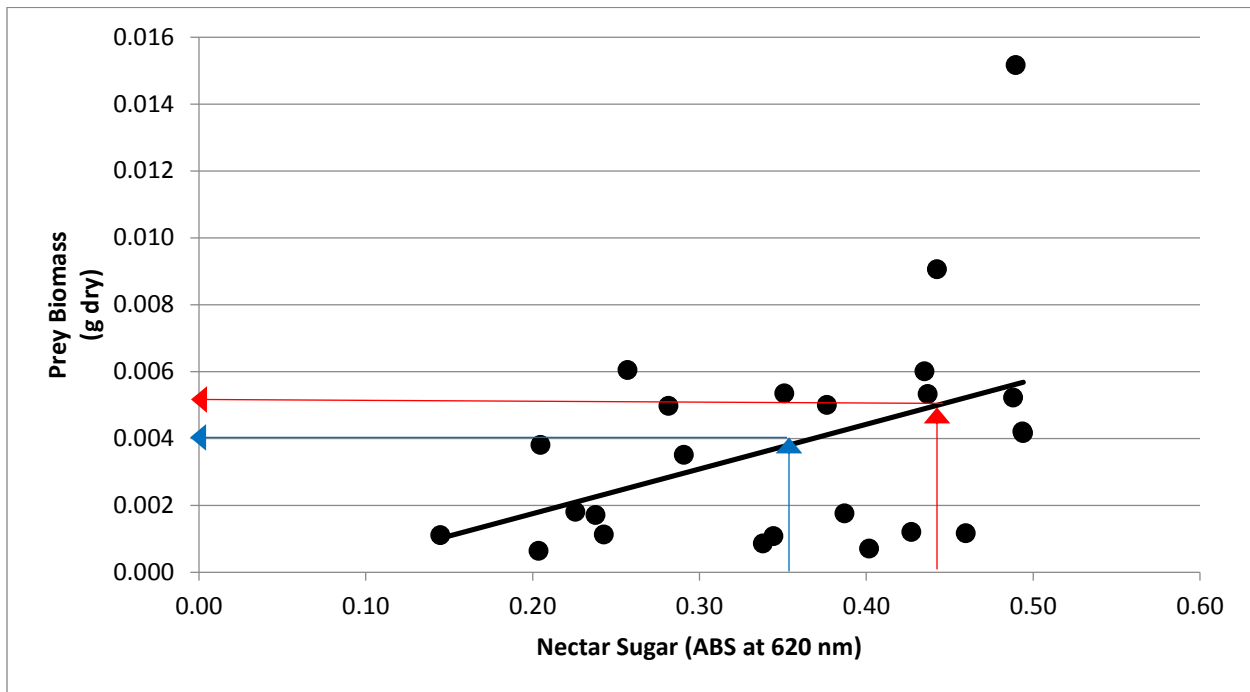


Fig. 11 Blue lines indicates current sugar and prey biomass relationship. Red lines indicates future predicted sugar and prey biomass relationship with a 1.2 milligram increase.

We also provide preliminary calculations as a way to infer inquiline abundance: A 27% increase in C:N and nectar sugar would result in pitchers containing an additional 5.5 more mosquito larvae and 1.5 more midge larvae totaling 7 additional inquilines per pitcher plant. Finally, effects from fossil fuel burning and elevated CO₂ may have positive effects on pitcher plant and their communities.

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