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

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Abstract

Human activities such as fossil fuel burning and deforestation have contributed significantly to the increasing levels of CO_2 in the atmosphere since the industrial revolution. Under the IPCC A1FI emission scenario, we are expected to reach levels as high as 1000 ppm by the end of the century. On average, C_3 plants experience a 27% increase in carbon to nitrogen (C:N) ratio under elevated CO_2. As a result, C_3 plants typically exhibit an increase in carbon-based secondary compounds. Carnivorous plants are a rare type of C_3 plant that has adapted to survive in low nutrient soils. The insectivorous pitcher plant *Sarracenia purpurea*, is a special kind of C_3 plant that captures prey to obtain nutrients and provides an aquatic habitat for mosquito and midge larvae. This study focuses on the potential effects of elevated CO_2 on pitcher plant nectar composition, prey capture, and inquiline communities. We modified a technique using filter paper wicks to sample the nectar content of 24 pitchers in Mud Lake Bog, Pellston, Michigan. After our final nectar collection, we removed the fluid from each pitcher with a turkey baster augmented by syringe injection. We first counted and removed any inquilines from the pitchers and placed them separately in a petri dish, then we identified each prey head capsule using a dissecting microscope, then counted, weighed, and dried all contents for total prey biomass. Finally, we removed the lips from the 24 pitchers for C:N analysis. We used our results to address the following questions: 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?, and 3) Is pitcher C:N correlated with sugar content of nectar? Our data suggest that prey biomass, prey types especially mosquitoes and ants, inquiline abundance and C:N are positively correlated with the amount of nectar present on the lip of the pitcher. We therefore infer that future increases in C:N due to future increases in atmospheric CO_2 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 (CO_2) levels were fairly stable at approximately 280 parts per million (ppm) until 1850. Human activities such as burning fossil fuels and deforestation increased those levels to 380 ppm by early 2006. According to measurements taken at the NOAA Mauna Loa Observatory in Hawaii, the daily mean concentration of carbon dioxide surpassed 400 ppm in May of 2013 (NOAA 2013). 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 550–1000 ppm by 2100 (IPCC 2007).

For most plants, growth under elevated CO_2 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). Higher C:N results in increased carbon-based compounds such as total carbohydrates, starches and soluble sugars and secondary compounds not involved in primary metabolism such as total phenolics and condensed tannins (Robinson et al., 2012). Elevated CO_2 reduces nitrogen concentration of plant tissues, but had different effects on different plant types. Leaf nitrogen was reduced more in C_3 plants than in C_4 plants and N_2-fixers (Cotrufo et al., 1998). Stiling and Cornelissen (2007) found that C_3 plants also experience a 27% increase in C:N under elevated CO_2. Therefore, in the future plants are likely to have less nitrogen but more carbon available for important internal processes (Long et al., 2004).

Carnivorous plants derive some or most of their nutrients from trapping and consuming insects or other prey. Carnivorous plants have adapted to grow in places where soils are a very poor source of nutrients, especially nitrogen, and they typically grow in acidic bogs. There are over 670 species of carnivorous plants around the world (http://www.sarracenia.com/faq/faq1120.html). These plants are fascinating to most humans for their abilities to attract and digest prey. The four major types of carnivorous plants are fly traps, snap traps, bladder traps and pitfall (pitcher) plants (voices.nationalgeographic.com/.../25/carnivorous-plants-glow).

Pitcher plant leaves wrap into a “pitcher” or a vase like shape. In many species, pitchers contain both green tissue for photosynthesis and red tissue for attracting prey. The proportion of red tissue varies both within and between species. All 110 species of pitcher plants also possess extra-floral nectaries (EFN) (Juniper et al., 1989; Vogel 1998, Bennett et al., 2009). Nectar produced by

EFN on leaves, petioles, stipules, and stems contains both carbon-based sugars and nitrogen based amino acids to which prey insect are attracted (Baker et al., 1978; Dress et al., 1997; Deppe et al., 2000). Nectar production increases prey capture rates by attracting prey, and by decreasing friction on the pitcher lip (Bauer et al., 2009, Bennett et al., 2009), and thereby increasing the probability that prey fall into the pitcher. Once inside the pitcher, the slippery lip along with sharp downward pointing hairs makes it is difficult for prey to escape. Prey fall to the bottom of the pitcher where they drown in collected rainwater. Pitcher plants are considered to be passive in nature as they do not move; instead insects are attracted by the sweet nectar and the color of the plants.

Sarracenia purpurea, the northern purple pitcher plant (Fig. 1), is commonly found in bogs in the northern United States and Canada, but also occur in the panhandle of Florida (http://www.fs.fed.us/database/feis/plants/forb/sarpur/all.html). It is generally believed that *S. purpurea* lures its prey to its water-filled pitchers (Fig. 2) with a variety of traits including red color and sugary nectar, which is produced primarily on and around the lip (mouth) of the pitcher (Cipollini et al., 1994; Fig. 3). *S. purpurea* is also host to inquiline communities consisting of small aquatic invertebrates that spend part or all of their life cycle inside the pitcher (Harvey & Miller 1996). At least 165 species of inquilines have been identified from S. purpurea, including bacteria, mites, rotifers, protozoans, midge larvae and mosquito larvae (Adlassnig et al., 2011). Inquilines live in collected rainwater within pitchers and feed on captured prey or on other inquilines. Little work has been done to determine why ovipositing females chose particular pitchers; however, pitcher characteristics (i.e. nectar and/or color) (Nastase et al., 1995), proximity to other pitchers, surrounding vegetation and micro-climate (Heard 1994; Russel and Rao 1942; Clements 1963) may be influential.

Figure 1. Green and red pitcher

Figure 2. Fluid with captured prey

Figure 3. Nectar on the lip of a pitcher

Since elevated levels of CO_2 cause an increase in C:N in most plants, it is likely to do so in pitcher plants. Moreover, since higher C:N typically results in higher levels of carbon-based compounds future elevated levels of CO_2 may also cause an increase in the amount of sugar in the nectar of pitcher plants. If so, the continued burning of fossil fuels may enhance 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 CO_2 affect nectar sugar content, prey capture, and inquiline communities?

Materials and Methods:

Study Site:

This study was conducted in summer of 2013, at Mud Lake Bog, in Cheboygan County, Michigan. Maybe include a photo?

Initial Comparison of Nectar Collection Techniques

During our initial visit on July 1, 2013 we randomly selected eight pitcher plants for collecting nectar samples. Pitcher colors ranged from green to red with some variation in between. To determine the best method for collecting nectar from pitchers, we compared the wick-sampling techniques of McKenna and Thompson (1988) and Creswell (1993). Circular wicks were cut from Whatman Number 1 filter paper using a cork borer # 5 with a diameter of 10 mm. We applied a dry (unmoistened) wick to the left side of the pitcher lip with a vinyl paperclip for 30 minutes. On the right side of the pitcher lip, we applied a wet wick lightly moistened with deionized (DI) water and held it against the lip for 10 sec. We assumed the two sides of the pitcher lip contained equal amounts of sugar. After the appropriate time, each wick was removed and placed into an individual microcentrifuge tube, taken back to the lab, and frozen at -20^0 C until analysis.

We determined the sugar content of each wick by colorimetric anthrone assay modified for microliter volumes (Cipollini et al., 1994; Deppe et al., 2000). After preparing sugar standards (equal parts sucrose and fructose, with total concentration ranging from 1.22 to 12.09 mg/100ml), we extracted sugar from dry and wet wicks by vortexing for one minute in 3 ml MilliQ water. In separate screw-cap glass test tubes, we then combined 2 ml of sugar standard or wick extract with 4 ml of anthrone reagent (0.4 g in 200 ml concentrated sulfuric acid). After vortexing for 10 seconds, and the solutions were allowed to cool to room temperature. After 90 min, absorbance was read at 620 nm using a spectrophotometer (Cipollini et al., 1994), and sugar content was determined by comparison to the standard curve. After comparing the amount of sugar collected by the two methods, it was determined that the wet wick method was the best option for collecting sugar from pitcher.

Nectar sampling

After determining that nectar was present on the lips of the pitcher, that sugar was present within the nectar, and that the wet wick technique collected more sugar than the dry wick technique, we identified and sampled 25 pitchers that would be used in this study. On three separate dates (July 16, July 21, and July 28, 2013) each pitcher was bagged with bridal veil (supported by a wooden dowel and closed with a twist tie) to prevent insects from removing nectar from pitcher lips (Deppe et al., 2000; Wyatt et al., 1992). Twenty-four hours later, bridal veils were removed to collect nectar samples. To obtain the maximum amount of nectar, we wiped each lip with a wet wick on the inside of the pitcher lip ten times, on the outside of the lip ten times, and then across the top of the lip ten times.

Using a thermocouple, we measured the temperature of each pitcher lip both as just before the pitcher was bagged and just before the nectar was collected. After the third and final nectar collection, each pitcher was measured for size of opening (mouth), hood width, and tube length.

Prey collection and identification

After the final nectar collection, the fluid and the prey were removed from each pitcher with a turkey baster and a syringe injection. To ensure all insects were collected, we injected 10 cc of deionized water into the base of the tube of each pitcher. The contents were placed in 25 ml polystyrene sample collection cups, capped and numbered according to each pitcher, and taken back to the lab. We counted and removed each inquiline (Fig. 3) from pitchers and placed them in a separate petri dish. Each prey head capsule was counted and identified under a dissecting microscope. Prey sample containers were placed in a drying oven at ~70^0 F, allowed to dry for about 2 days, and then weighed.

A. B.

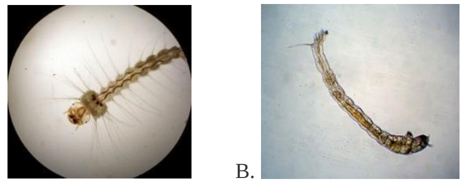

Figure 3. Images of inquilines A) Mosquito larva, B) Midge larva

Pitcher C:N ratio

On August 3, 2013, the lip was removed from each pitcher, placed in a small glassine envelope, numbered according to pitcher, placed in a large Ziploc bag inside an ice-filled cooler, transported to the lab, and placed in freezer at -20^0 C for 24 hours, then lyophilized for 48 hours. After freeze-drying, pitcher lips were ground under liquid nitrogen using a mortar and pestle. The ground lips were placed into numbered microcentrifuge tubes and placed in drying oven for ~15 min to remove water that condensed during grinding. Ground lip material was then analyzed for C:N using a mass spectrometer.

Statistical Analysis

The results of our analysis were determined by using regression and correlation analysis programs in both Microsoft Excel and SPSS.   Regression and correlation analyses were used to detect relationships between nectar sugar and prey biomass, prey types mosquitoes and ants, total number of inquilines, mosquito larvae, midge larvae, and C:N.

Results

Pitchers with more sugar contained more prey biomass, as indicated by a significant positive correlation between total prey biomass and nectar sugar content (R^2 = 0.18, d.f. = 23, p = 0.033; Figure 4). However, the relatively low R^2 value (nectar sugar only explains about 18% of the variation of prey biomass) suggests that other factors also affect prey capture. Prey biomass was also significantly or nearly significantly positively correlated with other factors such as size of lip length per cm (R^2 = 0.14, d.f. = 23, p = 0.07) and width of the pitcher mouth (R^2 = 0.33, d.f. = 23, p = 0.003). However, prey biomass was not significantly correlated with tube length or percent redness.

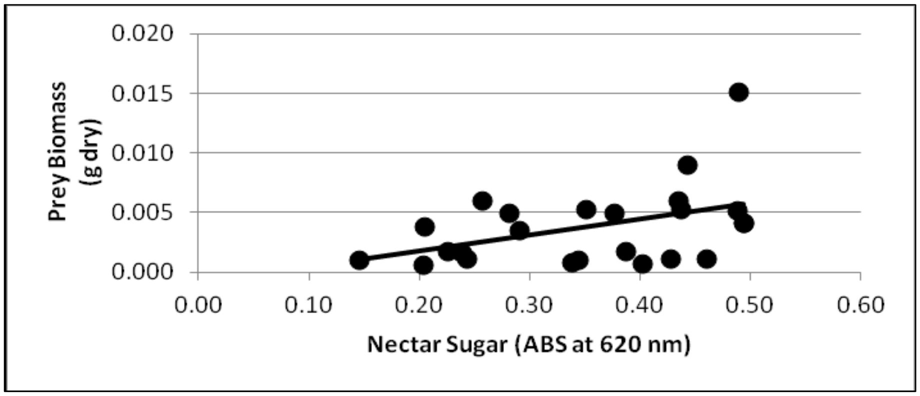

Figure 4. Relationship between nectar sugar (Abs @ 620 nm) and prey biomass in the pitcher. Each point represents one pitcher (n=24). The relationship is statistically significant and positive ($R^2 = 0.18$, d.f. = 23, p = .033).

A total of 118 prey individuals from nine prey types were identified from all 24 pitchers. Pitchers that produced more sugar captured more total prey individuals ($R^{2 =}$ 0.18, d.f. = 23, p = 0.038; Fig. 5a). Nectar sugar appears to be particularly attractive to ants ($R^2 = 0.18$, d.f. = 23, p = 0.034; Fig. 5b) and mosquitoes ($R^2 = 0.12$, d.f. = 23, p = 0.092; Fig. 5c).

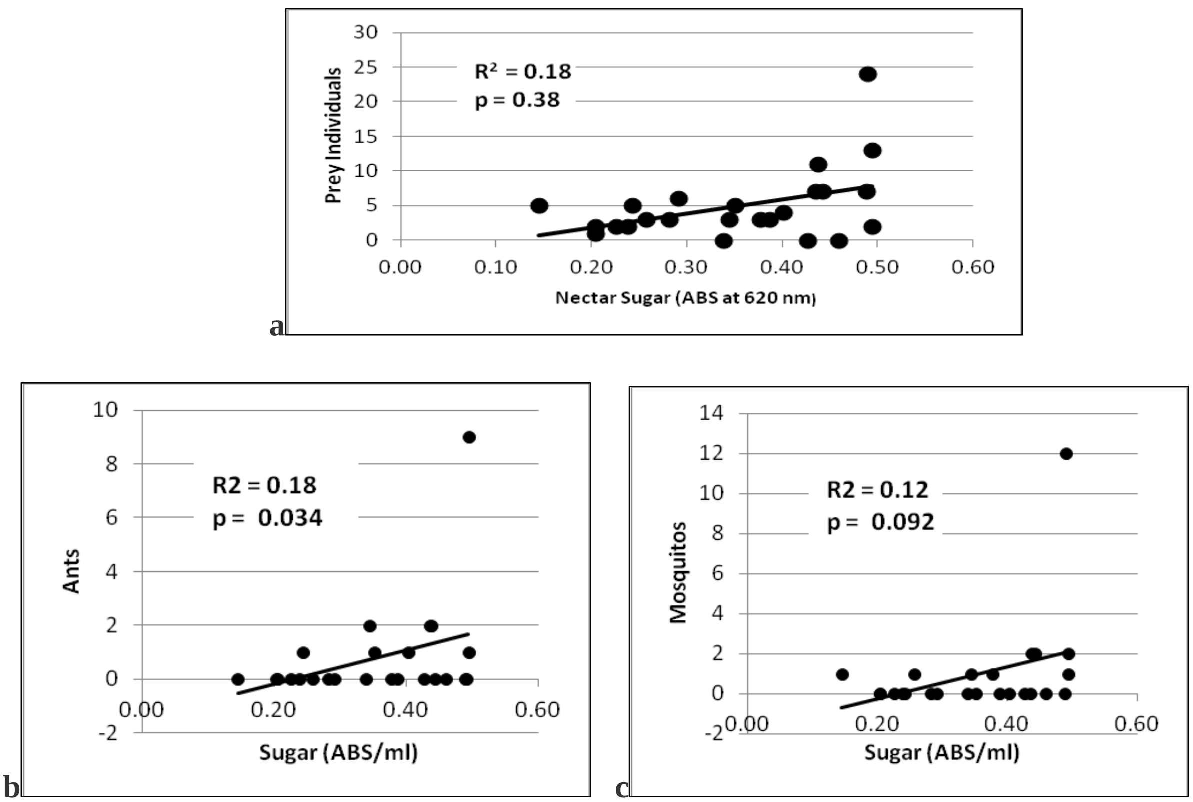

Figure 5. Relationship between nectar sugar (Abs @ 620 nm) and (a) total prey individuals ($R^2 = 0.18$, d.f. = 23, p = .038), (b) ants ($R^2 = 0.18$, d.f. = 23, p = .034), and (c) mosquitoes ($R^2 = 0.12$, d.f. = 23, p = .092) in the pitcher. Each point represents one pitcher (n=24). Each relationship is nearly or statistically significant and positive.

There were a total of 452 inquilines collected from 24 pitcher plants. We found that pitchers that produced more sugar contained more total inquilines (R2 = 0.26, d.f. = 23, p = .01; Fig. 9a). In particular, pitchers that produced more sugar contained more mosquito larvae (R2 = 0.20, d.f. = 23, p = .03; Fig. 9b).

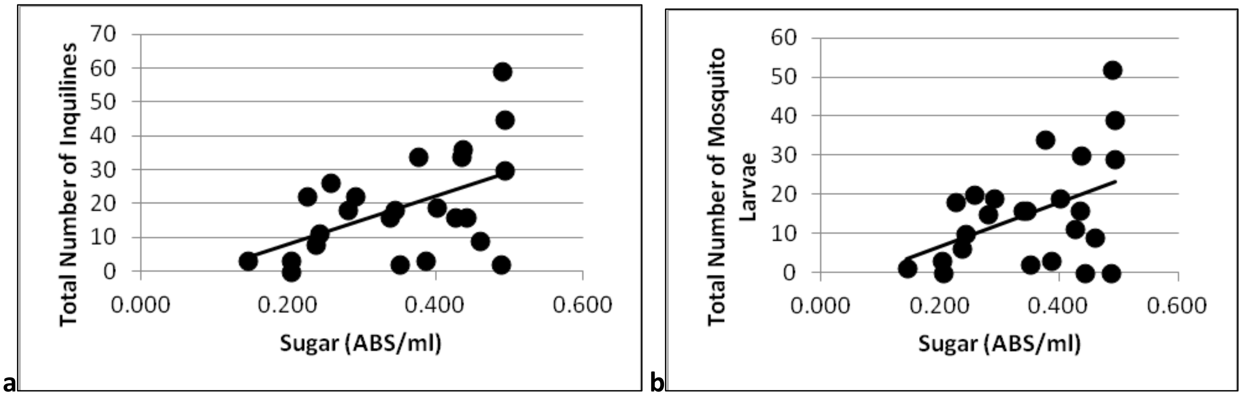

Figure 9. Relationship between sugar in nectar (Abs @ 620) and (a) total number of inquilines ($R^2 = 0.26$, d.f. = 23, p = .01), (b) mosquitoes ($R^2 = 0.20$, d.f. = 23, p = .03) in the pitcher. Each point represents one pitcher (n=24). The relationship is statistically significant and positive.

C:N ratio varied from 27.7 to 82.3 among the 24 pitchers in this study. Pitchers with higher C:N ratio produced nearly significantly more sugar ($R^2 = 0.14$, p = 0.067; Fig. 10).

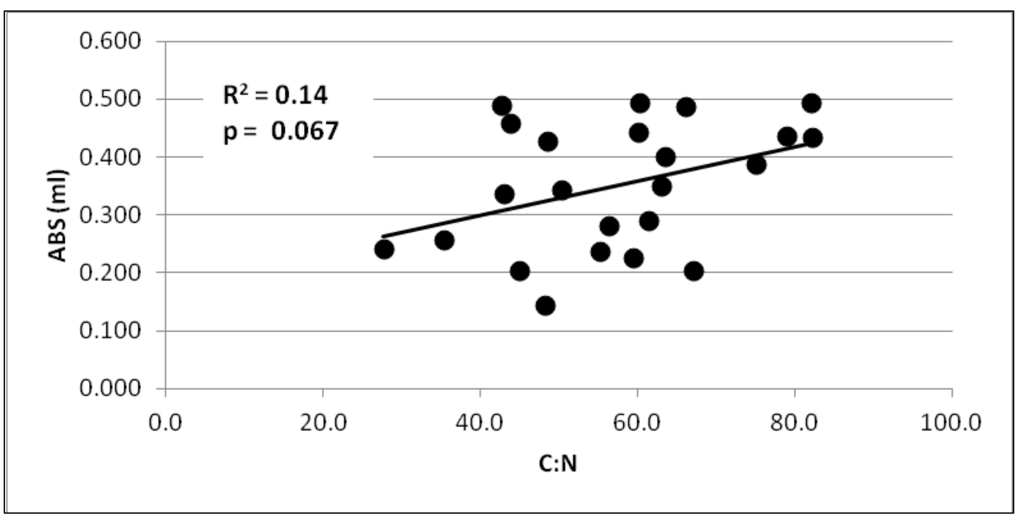

Figure 10. Relationship between C:N (x-axis) and sugar in nectar (Abs @620) in the pitcher. Each point represents one pitcher (n=24). The relationship is nearly significant and positive ($R^2 = 0.14$, d.f. = 23, p = .067).

Discussion

The results of our study indicate that pitchers with higher C:N produce nearly significantly more sugar and, probably as a consequence, capture more prey and contain more inquilines. . Since it is likely that a continued increase in atmospheric CO_2 will result in higher C:N ratios in pitcher plants, our research also suggest that in the future pitchers are likely to produce more sugar and, as a consequence, capture more prey and support a more abundant inquiline community.

When grown under elevated CO_2, C_3 plants typically grow larger because of increased carbon availability, but this results in decreased in leaf percent nitrogen. Higher C:N ratios of C_3 plants (on average 27% higher) lead to increases in carbon-based compounds such as tannins and other phenolics, starches, and non-structural carbohydrates including cellulose (Stiling and Cornelissen 2007; Zavala et al., 2013). Although we did not grow plants under elevated CO_2, we can use observed variation in C:N among pitcher plants today to infer the effect of further increases in C:N under elevated CO_2 on future prey capture and inquiline abundance (particularly mosquito and midge larvae). We observed the following relationships between nectar sugar (as absorbance at 620 nm) and prey biomass, mosquito larvae, and midge larvae (see also Figs. 4 and 9b):

Prey biomass = 0.0134*sugar – 0.0009

Mosquito larvae = 56.52*sugar – 4.57

Midge larvae = 13.79*sugar – 1.90

Assuming that pitcher plants will experience an approximately 27% increase in C:N, and that this increase will result in an approximately 27% increase in nectar sugar, we can interpolate to infer that future increases in atmospheric CO_2 will result in approximately 1.2 mg of additional prey biomass captured (Fig. 11), and also in approximately 5.5 more mosquito larvae and 1.5 more midge larvae, totaling approximately 7 additional inquilines per pitcher. Therefore, we tentatively conclude that elevated CO_2 from fossil fuel burning and deforestation may have beneficial effects on pitcher plants and their inquiline communities.

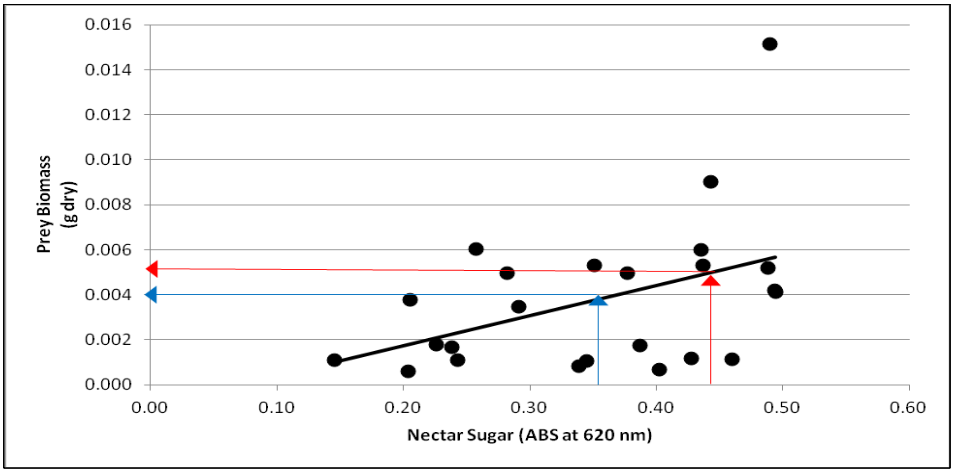

Fig. 11. Blue lines indicates current sugar and prey biomass relationship. Red lines indicates future predicted sugar and prey biomass relationship with a 1.2 milligram increase.

Our results suggest that nectar sugar plays a more important role than red color in attracting prey. Some previous studies found that color is the major attractant while others found that nectar is the primary attractant**.** Newell and Nastase (1998) found that, while the number of insect visits were not significantly correlated with pitcher size, potential prey were more likely to visit pitchers with higher levels of red venation. Schaefer and Ruxton (2008) showed that red color increases foraging success in carnivorous plants and overall capture rates of prey. In contrast, other studies have indicated that nectar may be a more important attractant than color. For instance, using artificial pitchers, Bennett and Ellison (2009) found that the number of ants captured was unrelated to the proportion of red color but was positively correlated with the presence of nectar. Cipollini et al. (1994) found that insects are drawn to the mouth of the pitcher trap due to greater densities of nectar glands. Visiting insects benefit from sweet nectar rewards provided by pitcher plants (Joel 1988) and in return prey is consumed by plants, and provide essential nutrients the plants are lacking. Bhattarai & Horner, in a 2008 study showed that prey biomass is significantly positively related to a number of biological traits including pitcher plant UV reflectance, odor and size.

However, some studies have found no evidence that nectar attracts prey. For instance, Green et al. (2007) and Creswell (1993) found no correlation between nectar sugar concentration and prey biomass captured by individual pitchers. Instead, morphological traits such as size were found to be positively correlated with prey capture. In other studies, factors such as age proved to be a determinant in the variation of prey capture rates. Heard (1998) found that capture rates peaked in the first few weeks of a pitcher's existence and then decreased by 50% by the third month. Although larger pitcher sizes are correlated with greater quantities of attractants for prey, younger pitchers tend to capture significantly more prey than older pitchers (Fish and Hall 1978; Wolfe 1981). Several others studies have found that olfactory senses play a major role in attracting prey (Juergens et al., 2009; Miles et al., 1975; Juniper et al., 1989; Jaffee et al., 1995). Insects have been found to be attracted to the newly opened floral scent as well as the decomposition odors of older pitcher plants (Juergens et al., 2009).

This study is one of the first to find a relationship between nectar sugar and inquiline abundance; pitchers that produced more nectar contained more inquiline mosquito and midge larvae. This is reasonable, given that sugar enhances prey capture. Since inquilines consume captured prey, natural selection should have favored ovipositing female mosquitoes and midges that prefer pitchers with higher nectar sugar, since those pitchers would provide better habitat for their offspring. Other co-founding variables such as pitcher characteristics like age, size of mouth opening and/or lip length, color variation and temperature may also be a factor and may result in the total amount and increase of prey captured, prey biomass and total inquiline abundance.

While elevated atmospheric CO_2 may be beneficial for pitcher plants, other aspects of climate change are likely to have a much more important, detrimental effects. Although our study did not address extreme climatic variation and its effect on the wetland environments where pitcher plants grow, it is likely that future increased droughts will adversely impact pitcher plants. In the United States, short-term seasonal droughts are expected to intensify while larger southeastern and southwestern areas in the U.S. (including the southern Great Plains) are expected to experience more long-term droughts (Georgakakos et al., 2014). Climate change will further exacerbate a wide range of risks to the ecology of the Great Lakes area (Pryor et al., 2014) including massive water level shifts, flash floods brought on by heavy precipitation events, and extreme drought. If so, it is likely that the detrimental climate effects of rising atmospheric CO_2 on pitcher plants will outweigh the beneficial effects identified in this study.

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