Influence of *Sarracenia purpurea* (Purple Pitcher Plant) Morphology on Prey Biomass and Inquiline Number

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Abstract

Nitrogen is a critical limiting nutrient for terrestrial plants, especially in the nutrient-deficient soil of *Sphagnum* bogs. The purple pitcher plant, *Sarracenia purpurea*, survives in nutrient-deficient bogs by capturing nitrogen from prey organisms. Inquiline organisms live inside pitchers and influence the amount of nitrogen available to the plant. Because pitcher plants obtain nitrogen from prey, it is essential that they attract prey organisms to their pitchers. We determined whether pitcher hood color and venation play a role in attracting prey organisms. We analyzed 47 pitchers from different plants for the mean green-red ratio of their hoods, total vein length, prey biomass, number of inquiline organisms, and insect-derived nitrogen. Pitchers with higher green-red ratios attracted more prey, and plants that captured more prey had more insect-derived nitrogen. Pitcher hoods with more venation attracted less prey. Inquiline organisms did not appear to influence the amount of insect-derived nitrogen in the pitcher plant. In spring, pitchers with a higher green-red ratio and less venation apparently have an advantage in attracting prey.

Introduction

Along with water and sunlight, nitrogen is one of the most important requirements for terrestrial plant growth. Nitrogen is often scarce in terrestrial ecosystems and is therefore a critical limiting nutrient for plants (Vance 2001). In terrestrial ecosystems, microbial communities decompose leaf litter. In doing so, they convert nitrogen to a form chemically accessible to plants through the soil (Agran and Bosatta 1988).

In bogs, the acidic environment created by *Sphagnum* moss inhibits the microbial decomposition processes that make nitrogen available to plants. Thus, bogs have nitrogen-deficient soil, which prevents most plants from growing (van Breeman 1995). The insectivorous plant, *Sarracenia purpurea* (also known as the purple pitcher plant), has evolved to thrive in nitrogen poor ecosystems by participating in both predatory and mutualistic relationships with arthropods. The plant consumes arthropods such as spiders and flies. The pitcher concurrently serves as home for a group known as inquiline, which help break down prey and make nitrogen bioavailable for the plant.

Purple pitcher plants vary in size and number of pitchers on the plant. The pitchers are tubular leaves adapted to catch rainwater as well as insects. They have green to red hoods with distinct reddish veins surrounding their openings to attract prey. Inquiline organisms live inside the pitcher liquid and include bacteria, protozoa, aquatic mites, mosquito, and midge larvae. Along with prey autolytic enzymes, inquiline organisms in the pitcher help break down prey to yield biologically available nitrogen for the plant (Hepburn *et al.* 1920). For example, midge larvae aid in prey decomposition by boring into the prey body, which helps release autolytic enzymes and insect-digesting bacteria (Bradshaw and Creelman 1984). Plants with inquiline communities absorb more nitrogen than plants without such communities. However, the inquiline communities in *Sarracenia purpurea* take up some nitrogen for their growth, reducing nitrogen content in the pitcher available to the plant.

Figure 1. Pitcher plants have colorful, hairy hoods that influence their interactions with insects.

Pitcher plants obtain nitrogen from two sources: the soil and from prey organisms. Animal tissues contain higher proportions of the naturally occurring ‘heavy’ isotope of nitrogen (^{15}N), so a pitcher plant’s relative abundance of prey derived nitrogen can be determined using the ratio between the two nitrogen isotopes, ^{14}N and ^{15}N. Higher ratios of $^{15}N/ ^{14}N$ in a pitcher plant indicate more nitrogen derived from prey (Foss-Grant 2008).

Differences in pitcher plant morphology, such as size, color, venation and nectar availability affect the total biomass and number of prey captured. Larger pitchers and pitchers that have hoods with more color variation consistently captured more prey (Cresswell 1993).

However, these pitcher characteristics account for less than half of the observed variation in the abundance of mosquito and midge larvae in pitchers (Nastase *et al.* 1995). Pitcher plant traits may still affect interactions with insects.

In this study, we investigated the relationship between *Sarracenia purpurea* color (green-red ratio, will be explained later) and venation (total vein length) of the hood on the amount of captured prey and its effect on inquiline community size. We speculate these relationships will have consequences on ^{15}N content of the pitchers (see Figure 2).

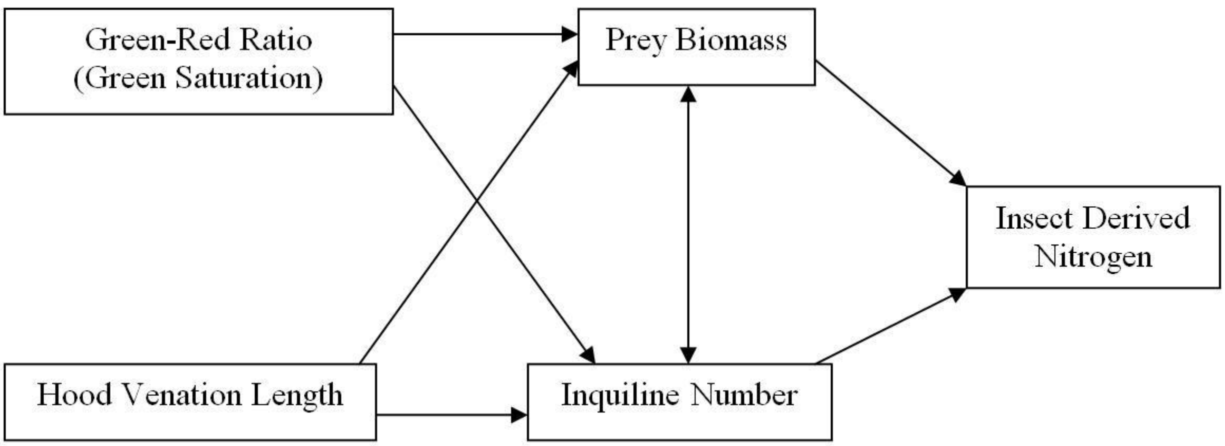


Figure 2. Pitcher plant traits may affect interactions with insects and ultimately insect derived nitrogen.

Specifically, we are testing:

1. Does green-red ratio and/or venation affect the amount of prey captured by the pitcher?

2. Does prey biomass influence the size of inquiline communities?

3. Is inquiline community size correlated with the amount of prey-derived nitrogen (^{15}N) in pitchers?

Materials & Methods

Study System

Our study site was Mud Lake Bog in Pellston, MI. We collected data for a week in mid-May. We selected 47 pitcher plants, of which we sampled the leftmost healthy pitcher to ensure randomness of color, venation, size, prey biomass and inquiline number of each pitcher.

Data Collection

First we photographed and flagged 47 pitchers around Mud Lake Bog. We removed the pitcher contents with a turkey baster, and placed the liquid into labeled glass vials. Then we removed an approximately 1 cm diameter sample of both the pitcher leaf and the nearby *Sphagnum*. We took field notes documenting location, size and number of surrounding pitchers for each sampled pitcher.

Effects of green-red ratio and venation length on prey biomass and inquiline number

To determine whether green-red ratio (G:R) affects the number of inquiline organisms in a pitcher, we first photographed the hood of each pitcher sampled. Using Adobe Photoshop Elements 8, we sampled 25 evenly spaced points on the photograph of each hood for the saturation value of red and green. To represent the relative amounts of red and green displayed by each pitcher, we found ratio of green saturation / red saturation. We then calculated the mean G:R for each hood, where hoods with higher G:R appeared greener and hoods with lower G:R appeared redder.

When analyzing the amount of venation on each hood, we converted each hood image to measure approximately 1000x1000 pixels. Using the program ImageJ, we traced the hood veins with line segments and the program calculated the total length of these line segments in pixels.

To determine the number of inquiline organisms per pitcher, we poured each sample into a Petri dish and looked at them through a dissecting microscope. The inquiline organisms in our samples were dominated by mosquito larvae and midge larvae, but mites were also present. We counted the number of each type of organism and removed them from the sample. We then regressed the number of inquiline organisms against hood color and total venation length to see whether hood color and/or venation attract inquiline organisms.

To determine the biomass of the prey in each pitcher, we removed the inquiline insects and plant matter and filtered the remaining sample with a vacuum pump. Then we freeze-dried the filtered prey remnants in the lyophilizer to obtain dry prey biomass. We weighed the prey remnants on the filter paper and subtracted the weight of the ‘cleaned’ filter paper to determine the prey biomass. We then regressed the prey biomass against hood color and total venation length to determine whether hood color and/or venation attract inquiline organisms.

The relationship between the amount of prey captured and inquiline number

We regressed the number of inquiline organisms against prey biomass to determine whether prey biomass influences the number of inquiline organisms in the pitcher.

Effect of inquiline organism community size on proportion of prey-derived nitrogen

We lyophilized the pitcher and *Sphagnum* samples, ground them to a fine powder and submitted them to Mike Grant for ^{15}N analysis. Then we regressed N^{15} against number of inquiline organisms per pitcher to determine whether inquiline community size influences prey-derived nitrogen.

Results

Relationship between green-red ratio and prey biomass captured

Pitcher prey biomass increases as the green-red ratio of a pitcher increases (R^2 =.15, df = 46, p = .008). This indicates that greener pitchers are more likely to have more prey biomass (Figure 3).

Does G:R ratio increase biomass?

Prey Biomass vs. G:R Ratio

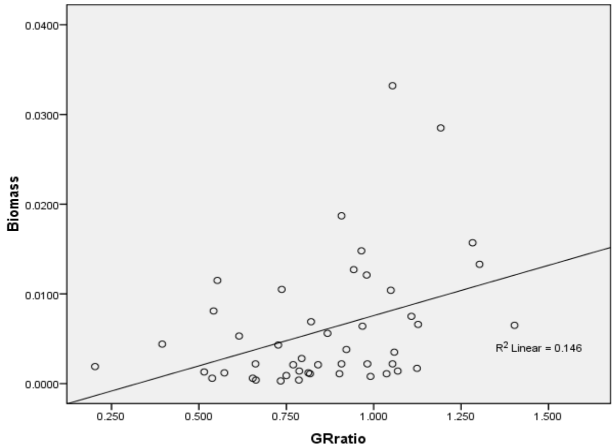


Figure 3 Shows a weak positive correlation between G:R ratio and prey biomass.

Relationship between venation and prey biomass captured

Prey biomass was nearly significantly negatively correlated with venation (R^2 =.08, df = 46, p = .052). This indicates that pitchers with greater venation are more likely to have less biomass (Figure 4).

Does venation increase prey biomass?

Prey Biomass vs. Venation

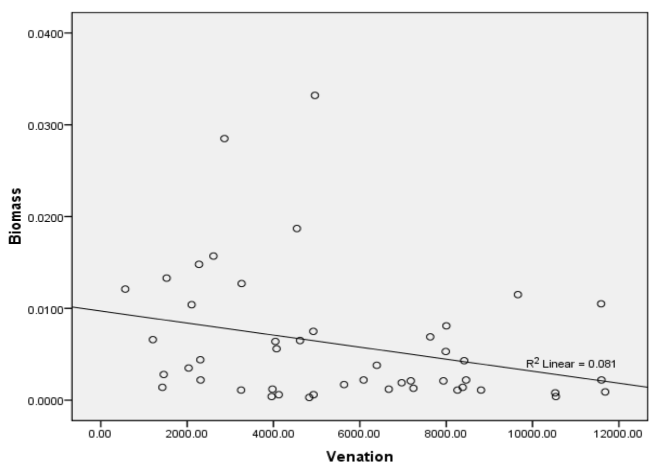


Figure 4 shows a weak negative correlation between venation and prey biomass.

Relationship between prey biomass and size of number of inquiline organisms

Inquiline community size was nearly significantly correlated with prey biomass ($R^2 = .07$, df = 46, p = .08). This indicates that as biomass increases, inquiline number increases (Figure 5).

Is prey biomass correlated with the number of inquiline organisms?

Inquiline number vs. Prey Biomass

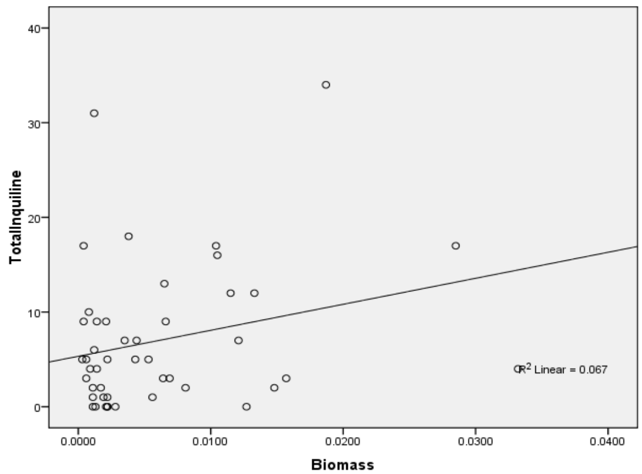


Figure 5 shows a weak positive correlation between inquiline number and prey biomass.

Relationship between prey biomass captured and ^{15}N

Prey biomass is nearly correlated with ^{15}N in pitchers (R2 = .15, df = 23, p = .066). This indicates more prey biomass will increase ^{15}N in pitchers (Figure 6).

Is prey biomass correlated with ^{15}N in pitchers?

^{15}N in Pitcher vs. Prey Biomass

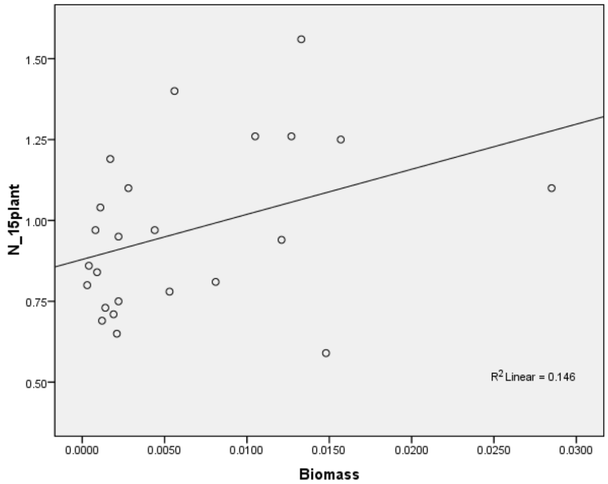


Figure 6 shows a weak positive correlation between prey biomass and ^{15}N in pitcher.

Relationship between green-red ratio and ^{15}N

We expect there to be an indirect relationship between the G:R and ^{15}N. G:R appears to significantly influence ^{15}N in pitchers (R^2 = .31, df = 23, p=.005) (Figure 7).

Is G:R ratio correlated with ^{15}N in the pitcher?

^{15}N in Pitcher vs. G:R Ratio

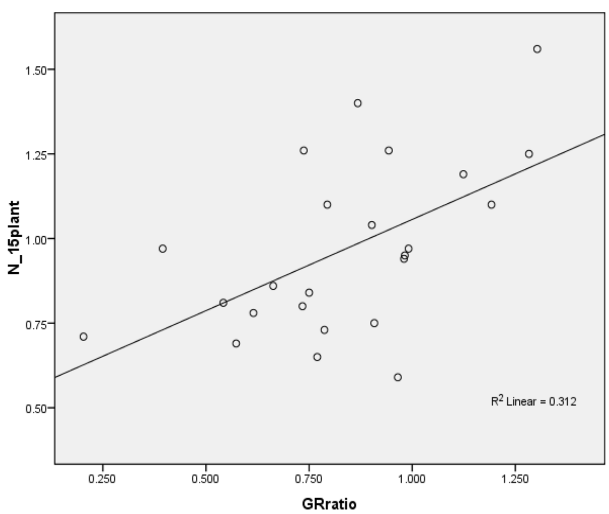


Figure 7 shows a relatively strong positive correlation between G:R ratio and ^{15}N in pitcher.

Relationship between number of inquiline organisms and ^{15}N

Number of inquiline organisms is not correlated with ^{15}N content in pitchers ($R^2 = .028$, df = 23, p = .437) (Figure 6).

Is inquiline community size correlated with ^{15}N?

^{15}N in Pitcher vs. Inquiline Number

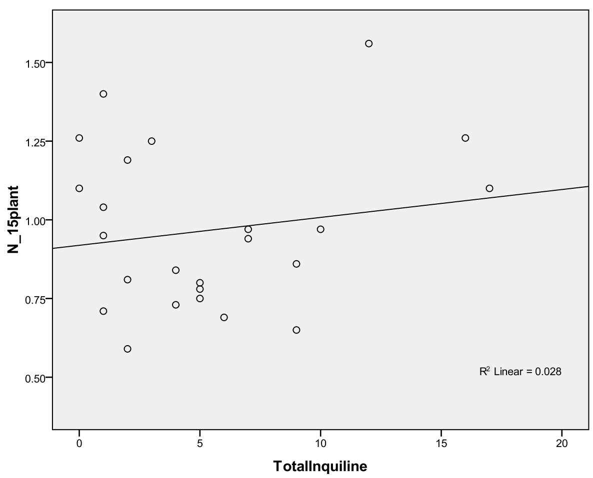
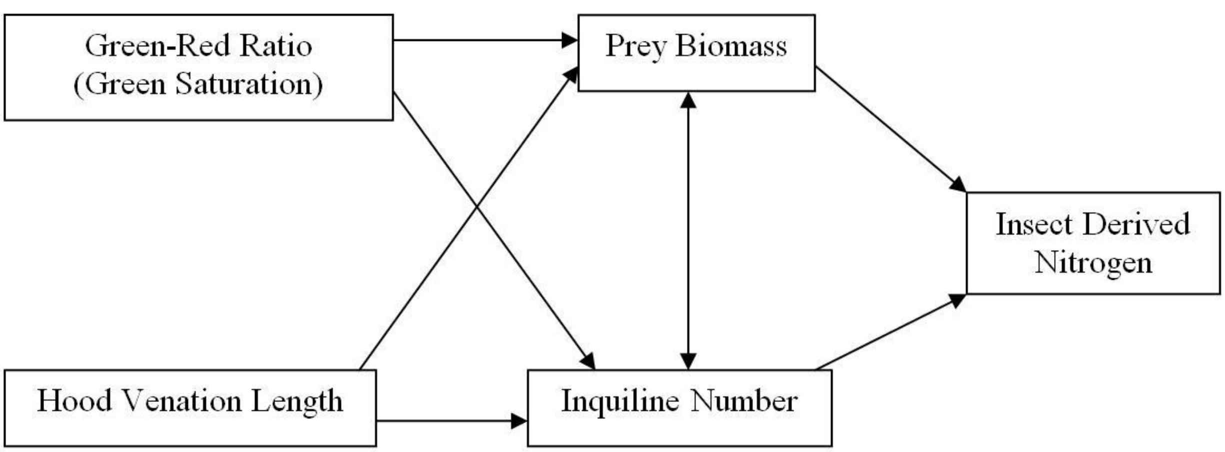


Figure 7: Illustrates the relationship between inquilines community size and ^{15}N.

Discussion



Green-red ratio (color saturation) effects on prey biomass captured

The significant increase in prey biomass with G:R ratio was surprising. We expected hoods with a lower G:R ratio to attract more biomass, as redder hoods more closely resemble typical insect attractants such as flowers or meat. Several possible explanations exist for our results. Younger pitchers tend to have paler leaves than older pitchers, and younger pitchers have higher success rates in capturing prey (Fish and Hall 1978, Cresswell 1993). We observed that green pitchers were paler than the deeper-colored red pitchers. We also noticed that most of the bog's damp *Sphagnum* had a dark red-purple color, so younger pitchers with paler leaves are more distinct against the *Sphagnum*. If prey find pitchers based on visual cues, it may be easier for them to locate greener pitchers.

It is also possible that the benefits of having a certain G:R ratio vary throughout the year. A study conducted in Mud Lake Bog from late July to August (as opposed to our study, which was carried out in mid-May) found that pitcher hoods with increased redness contained more prey biomass (Foss-Grant 2008). Our study took a 'snapshot' of the pitcher plant community without investigating interactions over time. The result that greener pitchers appeared to capture more insects may not be true throughout the year. Prey organism communities likely vary through the summer, and these varying prey organisms may have different visual cues.

There are several possible confounding variables that could affect our results, such as pitcher size. Pitchers with higher G:R ratios may be larger on average, which would show a positive correlation between color and biomass. To check that this was not the case, we ran a Kruskal-Wallis test between 3 groups of pitchers (small, medium and large) and color did not vary significantly from group to group (Chi-Square = 3.92, df = 2, p = .141).

Total venation length effects on prey biomass captured

An increase in hood venation length was nearly significantly correlated with a decrease in prey biomass. This finding was unexpected, since we thought increased hood venation served as a visual cue for attracting prey. Cresswell (1993) found increased venation correlated with an increase in prey capture, which opposes our results.

The near-significant correlation between increased hood venation and decreased biomass is not surprising when considering the positive correlation between G:R ratio and prey biomass. Veins are always red, so a hood with many veins would also have a lower G:R ratio, and therefore would likely have less prey biomass.

We observed that pitchers with less venation only had lateral veins, but few or no horizontal veins. If a decrease in venation length does serve as a positive visual cue, it is possible that hoods with only lateral veins better direct insects into the pitcher since they point into the pitcher.

Prey effects on inquiline community size

There was almost a significant positive correlation between prey biomass and inquiline community size in a pitcher. We expected a significant increase in prey biomass with inquiline community size, since it seems likely that mothers of inquiline insects and prey organisms respond to similar visual cues when approaching a pitcher.

There may have been a stronger positive correlation between inquiline community size and prey biomass when the larvae initially hatched. However, a large inquiline community would likely consume prey organisms quickly, which would decrease prey biomass in the pitcher. So a large inquiline community could diminish an initially significant positive correlation between inquiline community size and prey biomass.

Prey biomass effects on ^{15}N content in pitchers

For reasons we cannot explain, *Sphagnum* ^{15}N values were higher than those of the pitcher plants. Consequently, we could not determine the percentage of insect-derived nitrogen in the pitcher plant leaves. Instead, we analyzed insect-derived nitrogen by assuming the ^{15}N in the pitcher leaves came from insects. With this assumption in place, we found a nearly significant positive correlation between prey biomass and pitcher leaf ^{15}N.

Inquiline community size effects on ^{15}N content in pitchers

Inquiline organisms can make nitrogen biologically available to the pitcher plant through their waste, but they can also uptake nitrogen for their own growth. There is no significant correlation between number of inquiline organisms and ^{15}N in pitcher leaves. This lack of a significant correlation suggests that the inquiline organisms make nitrogen biologically available and take up nitrogen at approximately the same rate.

Green-red ratio effects on ^{15}N content in pitchers

Plants with higher G:R had higher ^{15}N content. This is due to an indirect relationship between G:R and ^{15}N content. As stated previously, plants with higher G:R capture more prey, and plants with more prey have a higher percentage of prey-derived nitrogen (^{15}N).

Another possible explanation for our results is that ^{15}N influences pitcher color. Pitcher leaves do not necessarily stay a constant color, since they have the ability to change their coloration from season to season (Schnell 1979). We found that green pitchers contain more nitrogen than red pitchers of similar size, so they are potentially less nitrogen limited. Plants with sufficient nitrogen may devote more resources to capturing carbon. Because chlorophyll is more efficient for photosynthesis, pitchers with sufficient nitrogen may produce more chlorophyll in their leaves, increasing their G:R ratio. So our conclusion that G:R affects ^{15}N content in pitchers may be just the opposite: ^{15}N content in pitchers affects G:R.

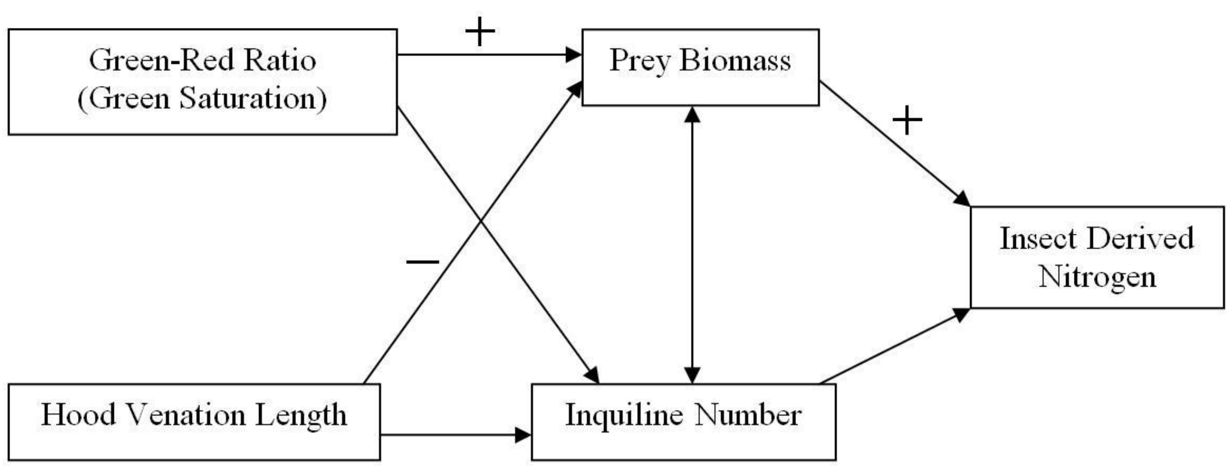


Figure 9. The table above summarizes our conclusions. Arrows with no indication of a positive or negative effect are interactions that either did not show a significant effect or we did not have evidence to accept the alternative hypothesis.

Limitations

One of this study's limitations was the error in the sphagnum nitrogen isotope analysis. Because nearly all the sphagnum samples had higher ^{15}N values than their corresponding pitcher plant sample, we could not necessarily make valid conclusions regarding percentages of insect-derived nitrogen in the pitcher plant.

When determining pitcher plant attractants, we only analyzed visual cues. Other possible attractants, such as nectar or chemical secretions, may play a larger role in attracting organisms to the pitchers. Consequently, these other possible attractants may be confounding variables in our study.

Future Work

Even though pitchers with a high G:R ratio were found to attract more prey organisms, we cannot be sure if the increased G:R ratio was the only factor attracting the prey. It would be interesting to determine whether green pitchers are linked with other advantageous characteristics for catching prey. For instance, perhaps greener pitchers tend to secrete a higher volume of nectar.

Another interesting possibility is that increased prey-derived nitrogen leads to an increase in green coloration on pitcher hoods, rather than increased green coloration attracting more prey organisms. Manipulating pitchers by adding prey biomass and/or pure ^{15}N and observing any change in coloration would address this possibility.

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