Digestive Rates of Old and New Leaves of *Sarracenia purpurea* without the Assistance of Diptera Inquilines

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Abstract - Previous research of the purple pitcher plant (*Sarracenia purpurea*) characterizes the effects of aging on its pitcher leaves, though to date there are no studies that characterize changes in plant-produced digestive enzymes in relation to age. This study looked at the digestive rates in 20 old and new pitchers with and without their Dipteran inquilines, in Mud Lake Bog, MI. Digestion of flies did not differ significantly between pitchers with and without the Diptera in both old and new leaves ($P>0.5$). During the course of the study the Diptera reoccurred in all but three pitchers, though digestion between old and new pitchers still did not differ significantly ($P>0.5$). These data suggest that there is no relative difference in digestive rate due to age, though this study could not fully account for purely plant-based digestion.

Introduction

The purple pitcher plant (*Sarracenia purpurea*) is a carnivorous plant, adapted to live in severely nutrient-limiting ecosystems, such as ombrotrophic bogs. The pitchers serve as a pitfall trap to insect prey. Nectar and red veinous coloration attract insects down into the sloping pitcher (Cresswell 1991). If the insect tries to escape, downward-pointing hairs and a waxy coating serve to minimize escape, though pitchers have low (i.e. <1%) capture efficiency (Newell & Nastase 1998). The insect prey is then drowned in a rainwater pool, where digestion is facilitated by the pitcher's symbiotic inquilines, as well as its own digestive enzymes (Robinson 1908). Approximately one tenth of a pitcher plant's annual nitrogen uptake is derived from insect prey (Chapin & Pastor 1995). The inquilines are therefore an essential factor in facilitating *S. purpurea*'s nutrient uptake in its already nutrient-deficient habitat.

S. purpurea's inquilines consist of bacteria, rotifers, and three predominant Dipteran larvae: the sarcophagid maggot *Blaesoxipha fletcheri*, the mosquito *Wyeomyia smithii*, and the midge *Metriocnemus knabi*. These larvae each occupy different layers of the pitcher water column and facilitate different steps in digestion (Fish & Hall 1978). *B. fletcheri* floats on the surface and is responsible for the initial breakdown of floating prey. *W. smithii* is a free-swimming filter-feeder and a keystone predator in managing bacteria richness, diversity, and abundance through top-down control (Cochran-Stafira & von Ende 1998; Peterson et. al. 2008). Lastly, *M. knabi* occurs at the base of the pitcher and facilitates the breakdown of insect husks since pitchers do not produce chitinase (Gallie & Chang 1997).

Inquiline respiration and pitcher photosynthesis are complementary systems (Bradshaw & Creelman 1984). Under higher temperatures and light levels pitcher photosynthesis and inquiline respiration are elevated. Besides providing inquilines with food and shelter, pitchers actively oxygenate their water, and remove CO_2, ammonia, and other metabolic byproducts from

the inquilines' habitat. Thus, the inquilines have a stable environment as long as they provide the nutrients for pitcher function.

As pitchers age, their capture success and inquiline composition change drastically. Newly opened pitchers have the highest capture efficiency within their first 30 days (Fish & Hall 1978; Wolfe 1981). A decrease in the pH of pitcher fluid complements the slow decomposition of prey as the pitchers age (Fish & Hall 1978), i.e. the effects of aging are synchronous with digestion (assuming a young pitcher does have capture success).

Inquiline composition changes with pitcher age and stages of digestion of prey (Fish & Hall 1978). *B. fletcheri* only occur in new pitchers (less than 40 days old), and are only present during the preliminary stages of digestion when prey are intact and still floating. *W. smithii* occurs in large numbers in newly opened leaves, and persist as long as there is particulate matter present (Nastase et. al. 1995). Oviposition and abundance of *W. smithii* decreases with pitcher age. *M. knabi* occurs in greatest abundance after *B. fletcheri* and *W. smithii* populations have begun to decline (Fish & Hall 1978; Nastase et. al. 1995).

Lastly, as pitchers age, enzyme release transitions from a developmental regulation mechanism to a signal transduction mechanism (Gallie & Chang 1997). Developmental regulation occurs in newly opened pitchers and entails a constant release of hydrolases, even in the absence of water or prey. Hydrolase secretion in mature leaves is regulated by a signal transduction mechanism, whereby enzyme release is stimulated by the presence of protein and nucleic acids in the pitcher fluid. Whereas developmental regulation of enzyme secretion occurs in all newly opened pitchers, hydrolase secretion occurs independently in mature pitchers.

To date, existing literature on the effects of aging on pitcher leaves does not specifically focus on changes in the strength of plant-produced digestive enzymes. In an effort to describe

digestion by virtue of pitcher enzymes (and by bacteria and rotifer microinquilines), this study aims to determine the relative digestive rates of new and old pitchers without the assistance the Dipteran inquilines. We predict that young pitchers have stronger digestive enzymes by virtue of their developmentally regulated enzyme release, and therefore should exhibit faster digestion rates.

Materials & Methods

We selected ten pitcher plants along the North side of Mud Lake Bog (Inverness**,** Cheboygan, MI) following the tree line of the forest. Selected plants were chosen on the basis of number of pitchers (at least two) and separation (at least one meter apart). We then selected two pitchers from each plant based on relative size, so that a definitively smaller, younger pitcher (designated "new") could be compared with a relatively larger, older one (designated "old"). Size dimensions (pitcher circumference and height, and hood opening, width, and height) were measured to quantify the relative age difference (Figure 1).

Pitchers were equally divided into four categories based on age designation (old or new) and whether or not Dipteran inquilines were removed (inquiline-present or -absent). After treatment, each plant had either an old pitcher without Diptera and a new pitcher with Diptera, or an old pitcher with Diptera and a new pitcher without Diptera. We removed fluid from all pitchers with pipettes ("turkey basters"; Nastase et. al. 1991) and pooled it according to treatment group. Inquilines were strained out of the fluid of inquiline-absent pitchers with fine mesh, and were added to the pools of their corresponding inquiline-present pitchers (i.e. on the same plant). Pitcher fluid was then uniformly redistributed to pitchers within the four treatment groups. Diptera were randomly allocated among new and old inquiline-present pitchers. Lastly, a single

small fly (Calliphoridae sp. and Sciomyzidae sp.; Cresswell 1991) was placed in each pitcher and was allowed to be digested for seven days.

After one week, we retrieved all flies and assessed their digestion using a six-point subjective scale based on the presence of extremities and soft tissue (0=entire fly is missing; 1=fly is fully intact; 2=intact fly with some missing extremities and soft tissue; 3=fragmented body with substantial, but not total tissue loss; 4=fragmented body with all extremities and soft tissue gone; 5=fully digested, water contains husk fragments and odor/color indicative of decomposition). The number of Diptera per pitcher was also assessed after digestion (Figure 4; 5).

Independent-samples t-tests were used to analyze differences in digestion rates between old inquiline-present and absent pitchers, between new inquiline-present and absent pitchers, between new and old inquiline-absent pitchers, and between new and old inquiline-present pitchers. Paired-sample t-tests were used to look at digestion of old and new pitchers across all plants, and to look at differences in inquilines after the seven-day digestion period due to observations from the second sampling. All statistics were calculated using IBM SPSS Statistics software (version 19.0).

Results

Fly digestion in old inquiline-absent pitchers did not differ significantly from that of old inquiline-present pitchers (Figure 3; Independent t-test; P=0.882). Furthermore, there were no significant differences in fly digestion between new inquiline-absent and new inquiline-present pitchers (Figure 3; Independent t-test; P=0.38). Additionally, there were no significant differences between new and old inquiline-present pitchers (Figure 2; Independent t-test;

P=0.726), or between new and old inquiline-absent pitchers (Figure 2; Independent t-test; *P*=0.862). Paired sample t-tests showed no significant differences in fly digestion between all old and new pitchers (Figure 2; Paired-samples t-test; *P*=0.904), or between the number of Diptera post-treatment (Figure 4; Paired-samples t-test; *P*=0.698).

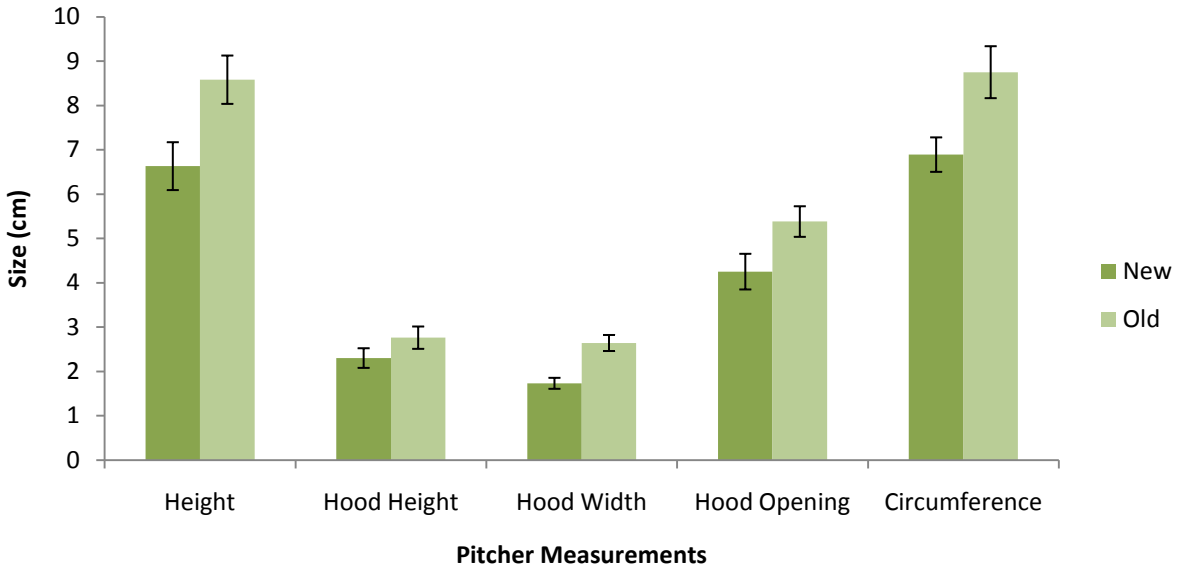

Figure 1. Size measurements for all new and old pitchers. Size measurements were used to quantify the relative age of pitchers. Smaller pitchers were designated "new," and larger pitchers were designated "old."

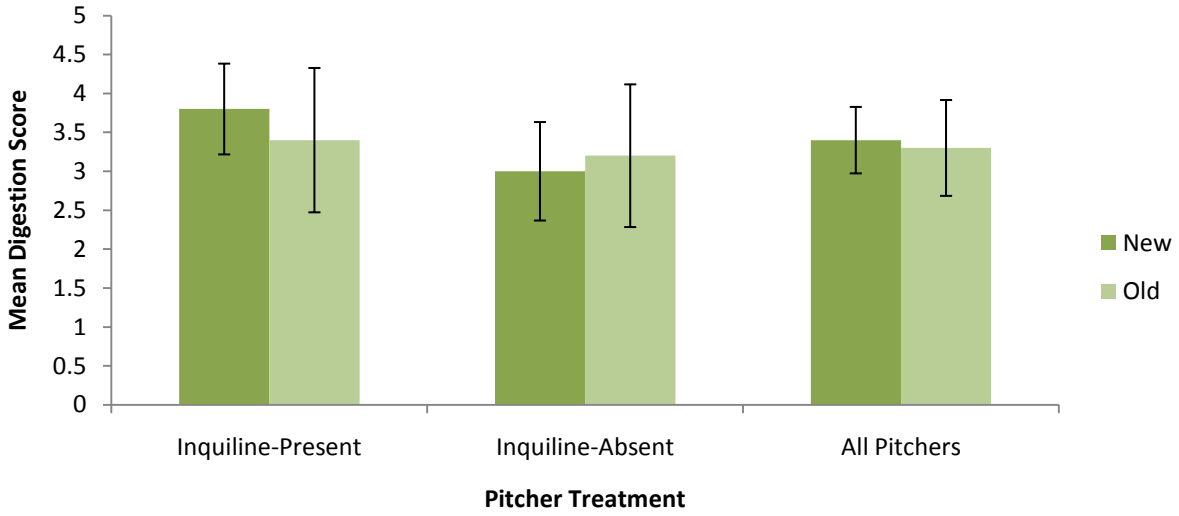

Figure 2. Mean digestion score (± S.E.) for old and new pitchers with inquilines, without inquilines, and across all old and new pitchers. Digestion did not differ significantly between inquiline-absent pitchers (Independent t-test; P=0.726), inquiline-present pitchers (Independent t-test; P=0.862), and old and new pitchers (Paired-samples t-test; P=0.904).

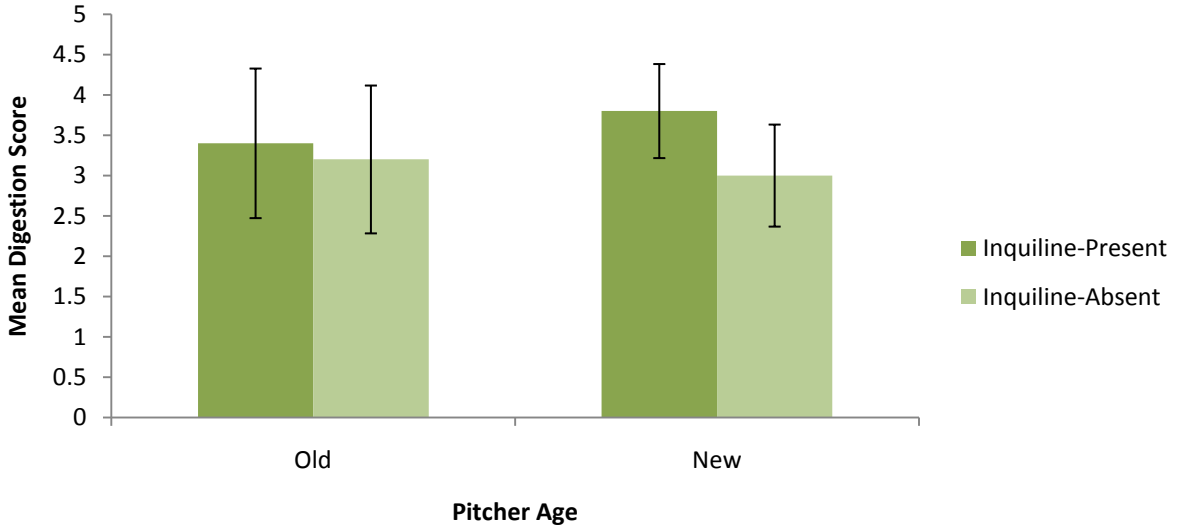

Figure 3. Mean digestion score (± S.E.) for old pitchers with and without inquilines, and new pitchers with and without inquilines. There were no significant differences between old pitchers with and without inquilines (Independent t-test; P=0.882), and between new pitchers with and without inquilines (Independent t-test; P=0.38).

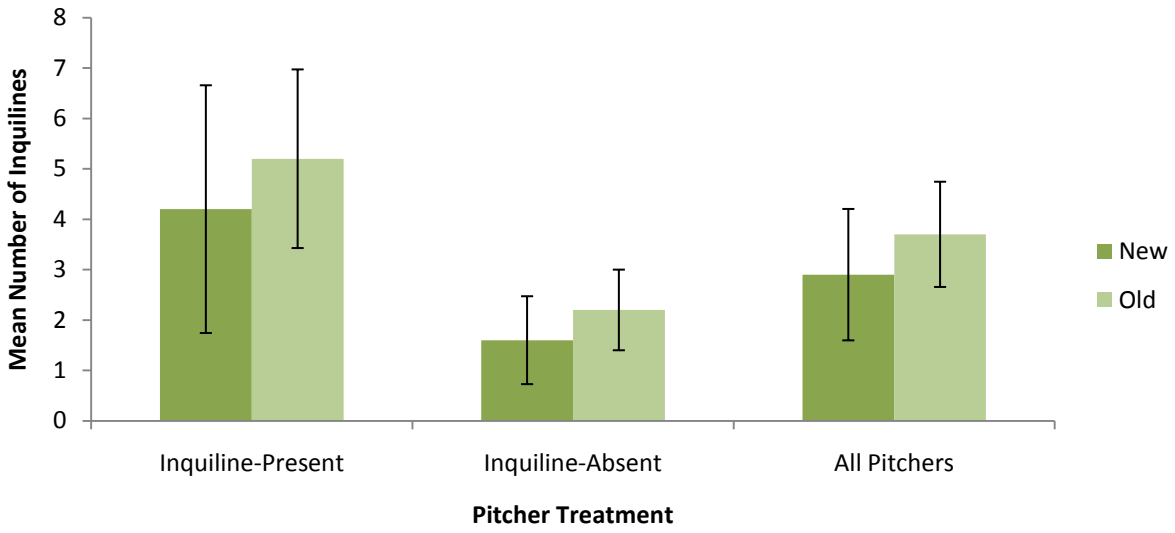

Figure 4. Mean number of inquilines (± S.E.) for old and new pitchers with inquilines, without inquilines, and across all old and new pitchers of inquilines. Though inquilines were removed from all inquiline-absent pitchers, they were found again in 17 pitchers after seven days. Inquilines did not differ significantly between old and new pitchers (Paired-samples t-test; P=0.904).

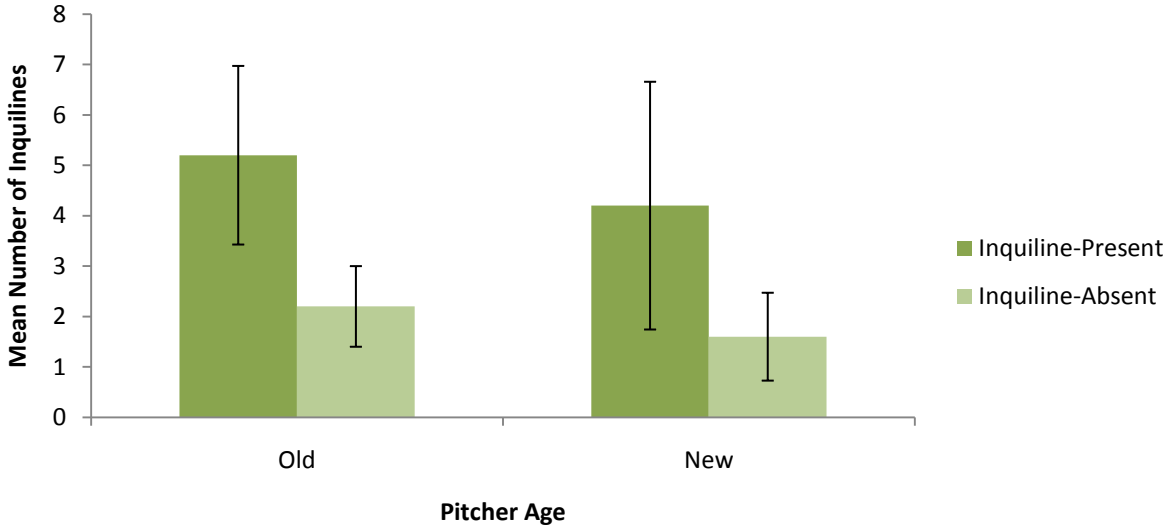

Figure 5. Mean number of inquilines (± S.E.) found in old pitchers (with and without inquilines) and new pitchers (with and without inquilines) after the seven day digestion period.

Discussion

Upon returning to Mud Lake Bog after seven days we found Dipteran inquilines in all but three of the pitchers (Figure 3). Inquilines were either not completely removed from pitchers due

to inefficiencies of our "turkey baster" method of removal (Nastase et. al. 1991), or adults oviposited during the digestion period. Since there was no way to determine at which point during the fly digestion the inquilines were introduced, we could not consider the extent to which the inquilines affected digestion. Therefore, our data only look at the differences in digestion rates between old and new leaves with all inquilines. However, in order to be consistent with our methodology, we still analyzed the differences within old and new pitchers, and between inquiline-present and inquiline-absent pitchers. We also tested for differences in digestive rates between all old and new pitchers (in following our new data), and for differences in the number of post-treatment inquilines. We found no significant differences between any of these groups.

Our data support the idea that the rate of digestion of young and new pitchers does not decrease with age. Furthermore, our data show that there was not a significant difference between the number of inquilines that might give some pitchers digestive advantages over others. Therefore, the data suggest that relative maturity does not affect the digestive rate, and therefore digestive strength, provided the number and digestive effect of all inquilines (i.e. Diptera, bacteria and rotifers) are similar among pitchers.

A possible explanation for why no significant differences in digestion rate were observed is that the experiment duration (seven days) was not long enough. In a study by Wolfe (1981), *Drosophila* sp. did not show significant signs of digestion in old and new *S. purpurea* leaves until at least 21 days. Additionally, a more effective and precise way to add flies and measure the rate of digestion would have been to measure their individual dry weight before adding them to the pitcher, followed by measurements of dry weight after digestion (Cresswell 1991). This would have allowed for a quantitative assessment of digestion, as opposed to the subjective

assessment utilized in our sampling. While this method is more precise, it is not exact, in that insect remains cannot be fully accounted for after digestion.

Our results can be explained by the "digestive life cycle" of a pitcher. New pitchers have higher capture efficiency than older ones, and either capture prey during this brief time period (i.e. a few weeks), or they do not (Fish & Hall 1978; Wolfe 1981). Regardless of whether they have caught an insect, pitchers are still secreting enzymes during their first few weeks (Gallie & Chang 1997). For those that have caught an insect, their digestive process and aging are synchronous; as pitchers age, pH decreases and changes in inquiline compositions compliment digestion. If a pitcher is in the process of digestion during the transition from developmental enzyme secretion to a signal transduction mechanism, enzyme strength need not be altered mid-digestion. However, this explanation can also support the study's initial hypothesis if the digestive enzyme were to decrease in strength as pH decreases (both facilitating digestion and denaturing the digestive enzymes) and inquilines accumulate to facilitate digestion.

Thus, if our data are not truly representative of the digestion in old and new pitchers, it might still be the case that the strength of the digestive enzymes secreted by younger pitchers is stronger than that of older pitchers. This study's hypothesis may still be accurate if enzyme potency is also developmentally regulated, i.e. if stronger enzymes are continuously produced in order to accommodate higher catch rates. Perhaps when the switch between developmentally regulated enzyme secretion and the signal transduction mechanism occur, enzyme strength is affected, weakening it. Since older pitchers have lower, more infrequent capture success it might benefit older leaves to produce weaker digestive enzymes since digestion is still facilitated by inquilines. In addition, older pitchers can release more enzymes over a greater surface area into their fluid. Thus, from a cost perspective, older empty pitchers would benefit from allocating

resources to other functions. Though this is only speculative, it provides a potential argument in favor of this study's hypothesis, and warrants future research.

Unfortunately, inquilines were able to re-enter the experimental set-up and render useless analysis of Diptera-absent versus Diptera-present groups. However, uniformity of inquilines between old and new pitchers led to the conclusion that the rate, and therefore strength of digestive enzymes does not significantly differ with age. If the strength of digestive enzymes can be considered constant between pitchers of varying age, this study has implications in researching the effects of individual inquilines on digestion. However, it should be noted that the removal of *W. smithii* may lead to changes in the diversity and abundance of microinquilines, which might have further effects on the rates of digestion (Cochran-Stafira & von Ende 1998; Peterson et. al. 2008).

In future support of the study's hypothesis, macroinquilines (e.g. Diptera), and bacteria and rotifers should be removed, and a similar experimental design should be run with more plants and a quantifiable method to measure digestion, such as dry weight of flies. Furthermore, relative maturity should be assessed on a more constant basis across all pitchers, such as Fish and Hall's (1978) methodology for determining relative maturity. Studies of this nature should be conducted in a laboratory, where inquiline composition can be controlled, and the specific age of pitchers can be documented. One might also consider a paired-statistics set up, where pitchers of a single plant have both had their inquilines removed or kept. Lastly, if a study of this nature is to be conducted in the field, it is essential that all inquilines are removed and effectively kept out (i.e. with some sort of cover).

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