A NOTE ON MUCILAGE AND HERBIVORE DAMAGE ON *BRASENIA SCHREBERI* IN A NORTHERN MICHIGAN LAKE

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ABSTRACT

Herbivory is an important part of aquatic macrophyte communities, in which the intensity of herbivory can impact the composition of macrophyte communities. The undersides of the leaves of Schreber's watershield, Brasenia schreberi, are covered with a thick, clear mucilaginous substance thought to reduce herbivory. Our study tested predictions of an earlier herbivore study by examining B. schreberi in situ and analyzing how herbivore damage correlates with the amount of mucilage found on the leaves. In September 2014, we sampled floating leaves in a small lake in Marquette County, Michigan. For each leaf sampled, we weighed the mucilage, measured the leaf, and estimated the amount of herbivore damage using Image J software. We found that the amount of herbivore damage on leaves differed significantly among the different herbivore larvae and with respect to leaves with no damage, which had the most mucilage. These results suggest that herbivores may be deterred from leaves with more mucilage and that different herbivores have different levels of tolerance to mucilage. Future studies should be conducted in which plants are grown in the laboratory, the mucilage is manipulated, and herbivore behavior in response to the amount of mucilage can be measured.

KEYWORDS: *Brasenia schreberi*, plant-insect interaction, aquatic macrophyte ecology, herbivory

INTRODUCTION

Herbivory is an important feature of aquatic macrophyte communities, where the intensity of herbivory can impact community composition with ecosystem-wide effects (Carpenter and Lodge 1986; Engelhardt and Ritchie 2001; Harms and Grodowitz 2009). For example, the golden apple snail (*Pomacea canaliculata*) invades wetlands throughout southeast Asia and removes aquatic macrophyte species, resulting in plankton blooms and increases in aquatic particulates (Carlsson et al. 2004). Herbivores can reorganize aquatic food webs, thereby altering fish communities and causing ecosystem-wide effects (Pípalová 2002; Dorn and Wojdak 2004; Miller and Crawl 2006). However, despite widespread knowledge about the impacts of herbivory on aquatic communities, little is

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known about specific plant–herbivore interactions of macrophytes and herbivores.

Brasenia schreberi J.F. Gmel. (Schreber's watershield) (Cabombaceae), is an emergent macrophyte widely distributed throughout North and Central America and eastern Asia (Elakovich and Wooten 1987). In Michigan, it is widespread in ponds and small lakes in quiet waters (Voss and Reznicek 2012). *Brasenia schreberi* is an aquatic perennial plant which produces leaves that float on the water surface. The undersides of the leaves are covered with a thick, clear mucilaginous substance secreted by trichomes. The mucilage, which is composed of polysaccharides, may be involved in defending against herbivores (McGaha 1952; Cronin et al. 1999; Dorn et al. 2001; Thompson et al. 2014). It is thought that this mucilage reduces the ability of insects to feed by interfering with their mouthparts or by inhibiting access to photosynthetic tissue thereby causing an obstruction to feeding (Thompson et al. 2014).

Twelve different species of insects have been identified as feeding on *B. schreberi* (Harms and Grodowitz 2009). These insects are representative of four families in three different orders: Chrysomelidae and Curculionidae (both Coleoptera), Chrionomidae (Diptera), and Pyralidae (Lepidoptera). Species in these families feed on a wide variety of aquatic macrophytes, and several members of Chrysomelidae, particularly *Donacia* spp., target macrophytes with floating leaves. These herbivores all have varied life histories and feeding habits that result in distinctly different feeding damage by each on host plants.

Although many insect species consume *B. schreberi*, the mucilage has only recently been shown to reduce insect herbivore feeding in general (Thompson et al. 2014). In a series of experiments, Thompson et al. (2014) scraped mucilage off individual *B. schreberi* leaves and recorded an increase of herbivore damage, showing that mucilage likely deters herbivores.

The objective of this study was to test the predictions of Thompson et al. (2014) as well as to look at *B. schreberi* damage *in situ* and analyze how this damage correlates with the amount of mucilage found on leaves (assuming that not all leaves have the same amount of mucilage).

MATERIALS AND METHODS

Leaves of *B. schreberi* were sampled at Harlow Lake, a property of the Michigan Department of Natural Resources, in Marquette County, Michigan in early September 2014 (Figure 1). In Harlow Lake, *B. schreberi* has a patchy distribution and is typically found within 30 m of the shoreline. We selected three patches of *B. schreberi* and, in each patch, sampled along one 20 m transect perpendicular to the shoreline. Using the point center quarter method, we sampled a total of 228 floating leaves. We selected leaves that showed signs of herbivory and leaves that did not show signs of herbivory. Leaves with no herbivory were selected so that we could compare the amount of mucilage on those leaves to leaves with herbivory. Leaves were cut from the stem and stored in a labeled bag. Samples were brought back to the laboratory immediately after sampling.

Sampled leaves were patted dry and photographed on a brightly colored background with a Sony Cybershot camera (14.1 megapixels). Areas of the background that showed through the leaves were considered areas of herbivore damage (Thompson et al. 2014). Mucilage was then removed from leaves using a razor blade and immediately weighed. The leaf images were manually edited using Adobe Photoshop Elements 9 to enhance the contrast between undamaged and herbivore damaged areas of the leaf (Figure 2). The public domain software Image J (Version 1.48) was used on these photographs to estimate the total leaf area and the percentage of the total area that was damaged by

FIGURE 1. Sampling at Harlow Lake, Marquette Co., Michigan. Leaves of *Brasenia schreberi* are visible around the canoe and dominate the submerged zone. Photo by Kevin Heynig.

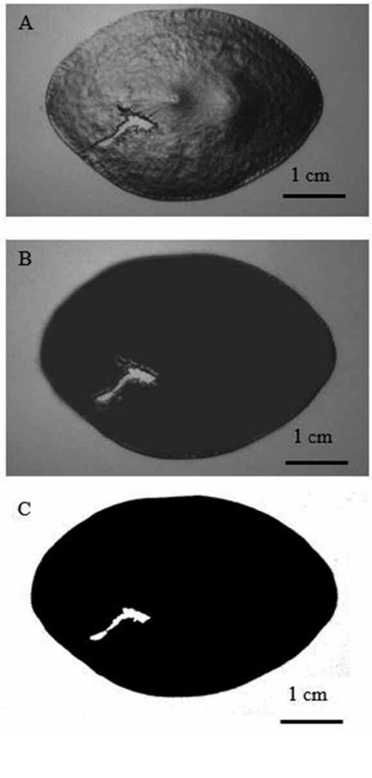

FIGURE 2. Representation of editing watershield photos to better estimate herbivore damage. (A) raw photo of watershield; (B) enhanced contrast between undamaged areas and herbivore damaged areas in Adobe Photoshop; (C) end result of photo in Image J. The increased contrast permits a better estimation of leaf area and herbivore damage measurements.

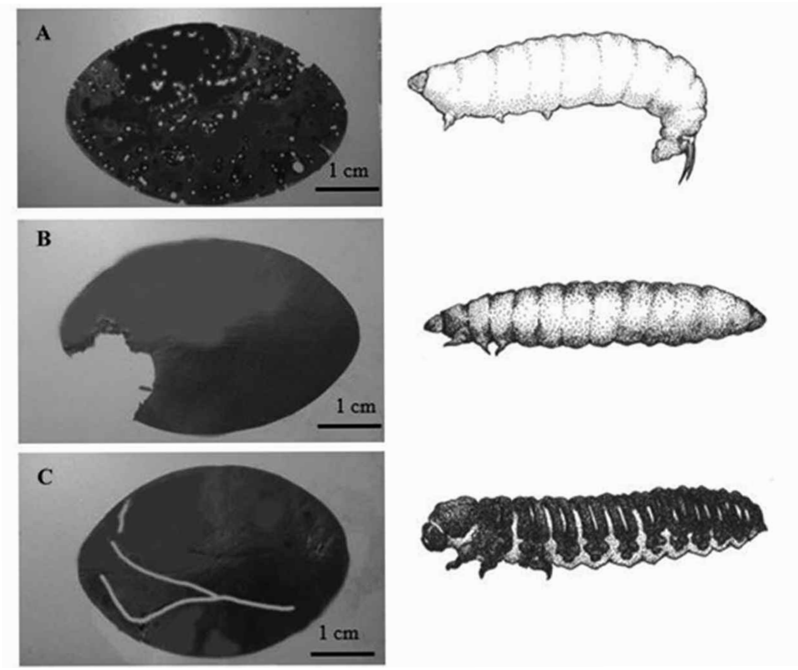

FIGURE 3. Common herbivores of *Brasenia schreberi* and examples of damage caused to leaves by each. A) The leaf beetle (*Donacia* spp.) (Coleoptera: Chrysomelidae) creates small random bite marks; B) the aquatic moth (Lepidoptera: Pyralidae) cuts out large contiguous areas for leaf shelters; and C) the waterlily leaf beetle (*Galerucella nymphaeae*) (Coleoptera: Chrysomelidae) leaves long linear mines through the leaf surface. Larvae illustrations by Jen Koppin.

herbivores. Areas of herbivore damage were visually assessed, and the pattern of damage was assigned to a specific herbivore based on previous research and known feeding habits of each of the herbivores (Merritt et al. 2008, Thompson et al. 2014). Although there is some overlap in the feeding habits of the different herbivores on the leaves and therefore a slight amount of pseudoreplication, we believe that the insects responded differently enough that this did not bias our results (Oksanen 2001). In our leaf samples, we detected herbivore damage representative of three types of aquatic larvae. These were aquatic moths (Lepidoptera: Pyralidae) and two beetles, the waterlily leaf beetle (*Galerucella nymphaeae*) and the leaf beetle (*Donacia* spp.) (both Coleoptera: Chrysomelidae) (Figure 3). We used a one-way ANOVA to test for differences in herbivore damage on leaves, and a Tukey's Multiple Comparisons test for pairwise comparisons among herbivores in R (3.3.2).

RESULTS

The amount of herbivore damage on leaves differed significantly among the herbivore larvae and with respect to leaves with no herbivore damage (ANOVA: $F_{1,\ 196} = 13.84$, $p=0.038$). Tukey HSD post-hoc analysis showed that herbivore damage to leaves by the aquatic moth larvae differed significantly from the her-

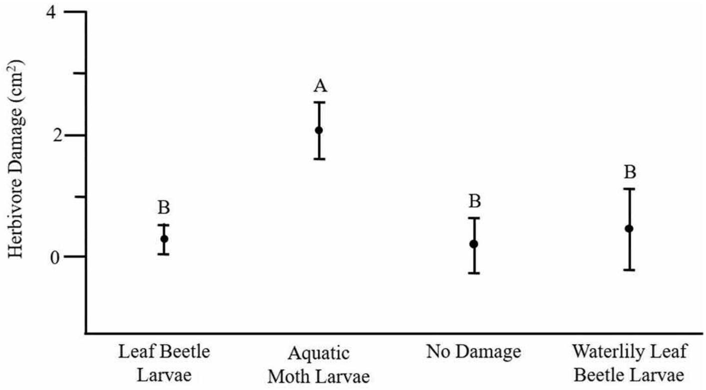

FIGURE 4. Results of one-way ANOVA and of Tukey's Multiple Comparisons test for pairwise comparisons among herbivore damage by the leaf beetle, the aquatic moth, and the waterlily leaf beetle larva, and leaves with no damage. Leaves with damage by the aquatic moth larvae differed significantly from leaves with damage from the other herbivore larvae and from leaves with no damage ($p < 0.05$). Different letters indicate significant differences between herbivores.

bivore damage to leaves by other larvae and from leaves with no herbivore damage ($p < 0.05$, Figure 4). In addition, leaves with no herbivore damage (0.0 cm^2) had the greatest amount of mucilage (0.76 ± 0.5 g), and leaves with high amounts of damage (2.03 ± 3.20 cm^2) had lesser amounts of mucilage (0.46 ± 0.41 g). Leaves with a moderate amount of mucilage were exposed to the greatest herbivore damage by the aquatic moth larvae, whereas leaves with the least amount of mucilage had the most herbivore damage to leaves by the two beetle larvae (Table 1).

DISCUSSION

The results indicate that aquatic moth larvae are able to tolerate greater amounts of mucilage than the leaf beetle larvae or the waterlily leaf beetle larvae. Beetles chew through the whole leaf, thereby being exposed to mucilage in almost all of their feeding. In contrast, adult aquatic moths lay their eggs on the

TABLE 1. Average leaf area, average herbivore damage, and average mucilage weight, with standard deviations of each, for leaves with herbivore damage on *Brasenia schreberi* by each of the three larval herbivores—aquatic moth larva, waterlily leaf beetle larva, and leaf beetle larva.

Herbivore Type	Avg. Leaf Area (cm^2)	Avg. Herbivore Damage (cm^2)	Avg. Mucilage Weight (g)
Aquatic Moth	38.2 ± 13.6	2.03 ± 3.20	0.46 ± 0.41
Waterlily Leaf Beetle	47.9 ± 15.4	0.57 ± 0.46	0.19 ± 0.18
Leaf Beetle	43.4 ± 13.8	0.35 ± 0.43	0.32 ± 0.36
No Damage	37.5 ± 13.2	0.00	0.76 ± 0.50

top of emergent shoots where there is little mucilage (Merritt et al. 2008). The moth larvae then hatch and chew the leaves from the top and "flip" the leaf over themselves as a shelter, thus avoiding areas with the most mucilage. These moth larvae then feed inside, eating parts of the leaves free of any mucilage.

Herbivory on *B. schreberi* is varied, and undoubtedly damage by other herbivores or by other factors could not be accounted for by our sampling. Even if herbivores such as moose or muskrats were influenced by mucilage, their feeding would completely remove plants and we would have missed their impacts. It is also possible that mucilage plays a role in allelopathy and competition among other aquatic macrophytes in addition to its role as a defense against herbivory (Elakovitch and Wooten 1987).

Environmental variables such as water depth or distance from the population to shore could also influence the macrophyte community and the herbivores present in the study area. Aquatic herbivores can be influenced by water depths and may therefore be absent from areas of greater depths (Aroviita and Hamalainen 2008). Turbidity and water chemistry also impact plant growth, and this can in turn impact the production of defense traits with ripple effects on insect herbivory.

In order to further examine the role that mucilage plays in the ecosystem, manipulative studies should be pursued. Studies in which *B. schreberi* is grown in the laboratory and exposed to herbivores would facilitate the direct observation of insect behavior. Studies throughout the extensive intra-continental range of *B. schreberi* would allow comparative tests of selection pressures on variation in the amount of mucilage on leaves. *Brasenia schreberi* and its associated insect herbivores provide an interesting system for many disciplines studying ecosystem interactions in aquatic food webs.

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