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Potential Role of Phenolics in Eurasian Watermilfoil (Myriophyllum spicatum) Invasion Success

Annelise Slack

LaTina Steele

Sara Zaytoun

POTENTIAL ROLE OF PHENOLICS IN EURASIAN WATERMILFOIL (*MYRIOPHYLLUM SPICATUM*) INVASION SUCCESS

Annelise Slack, Sara Zaytoun, and Dr. LaTina Steele

Department of Biology, Sacred Heart University, 5151 Park Ave., Fairfield, CT 06825

Sacred Heart University
Department of Biology

Abstract

Although the detrimental effects of invasive aquatic plants like Eurasian watermilfoil (*Myriophyllum spicatum*) are well documented, the factors leading to successful aquatic plant invasions are poorly understood. High levels of chemical feeding deterrents in invasive plant species may be at least partially responsible when invasive plants overgrow and dominate the invaded community. To investigate the role of phenolics in Eurasian watermilfoil invasions, whole *M. spicatum* (invasive) and *Ceratophyllum demersum* (native) plants were collected from Osbourndale Pond in Derby, Connecticut during September 2016 and frozen at -80°C until phenolic analysis. Colorimetric assays were used to measure total phenolic and condensed tannin content of the two plant species. A choice feeding experiment was conducted to determine if phenolics produced by *M. spicatum* affected feeding by amphipods, the dominant herbivore at our study site. Artificial diets were prepared by incorporating palatable fish food into an agar matrix and pouring the agar food over window screen. The control diet contained only a palatable food, while the gallic acid diet contained the palatable food plus gallic acid at a concentration common in *M. spicatum* extracts. Amphipods were simultaneously offered the control food and the gallic acid treated food. The number of squares that were cleared of food after 48 hours was recorded. Our results suggest that small phenolic compounds such as those measured by the Folin-Denis assay may play a role in successful milfoil invasions but that larger compounds like condensed tannins do not. Further study is needed to fully determine if these compounds may reduce consumption of milfoil.

Introduction

- Eurasian watermilfoil (*Myriophyllum spicatum*) has invaded many freshwater systems nationwide, but factors leading to successful milfoil nvasions are poorly understood.
- Plant invasions may succeed, in part, due to invasive plants' ability to produce allelopathic compounds that give them a competitive advantage over native plants[1].
- Phenolics are well-known feeding deterrents in many plants[2] and may lead to overgrazing on native species if invasive plants produce more phenolics than native species.
- Although some studies have examined phenolic production in invasive milfoil, to our knowledge, no studies have compared phenolic production in milfoil to that of the native *Ceratophyllum demersum*, a common species at our study site.
- This study tested the hypothesis that phenolics influence *M. spicatum* invasion success in ponds dominated by *C. demersum* by accomplishing the following objectives:
  1. Compare production of different sized phenolic compounds (large condensed tannins and smaller phenolics) in native *C. demersum* and invasive milfoil
  2. Assess the effects of gallic acid (a small phenolic compound present at a concentration of ~0.1% w/v in milfoil extracts) on feeding by a locally abundant amphipod (*Hyalella azteca*)

Methods

Plant Chemical Composition

- Invasive *Myriophllum spicatum* (Fig. 1) and native *Ceratophyllum demersum* (Fig. 2) were collected from Osbourndale Pond in Derby, CT, cleaned with spring water, and frozen at -80°C for later phenolic analysis.
- Condensed tannins and total reactive phenolics were extracted from frozen plant tissues using 1 mM ascorbic acid in 70% acetone.
- Concentrations of condensed tannins and total reactive phenolics were measured using two separate colorimetric assays (a butanol:HCl method for condensed tannins and a Folin-Denis assay for total reactive phenolics; Figs. 3 & 4).

Choice Feeding Experiment

- Control (palatable food only) and treated (palatable food with 0.1% w/v gallic acid) artificial diets were prepared by incorporating palatable fish food into an agar matrix and pouring the agar food over 1 in. x 2 in. squares of pre-cut and cleaned fiberglass window screen .
- One piece of control food and one piece of treated food were placed into each of 10 experimental chambers that contained 10 amphipods (*Hyalella azteca*, starved for 24 hours) each.
- The number of squares cleared of food was recorded after 48 hours.

Figure 1. Invasive *Myriophllum spicatum*

Figure 2. Native *Ceratophyllum demersum*

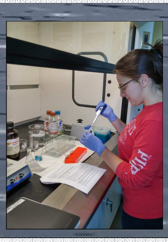


Figure 3. Conducting condensed tannins assay: mixing 125 µl of each sample extract with 1 ml of 95:5 butanol:HCl.

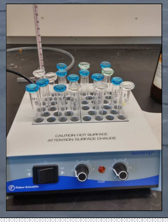


Figure 4. Condensed tannins assay: samples in butanol:HCl mixture heated at 100°C for 1 hour, causing condensed tannins in the samples to turn red.

Results

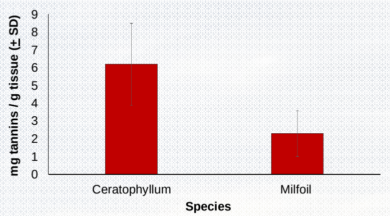
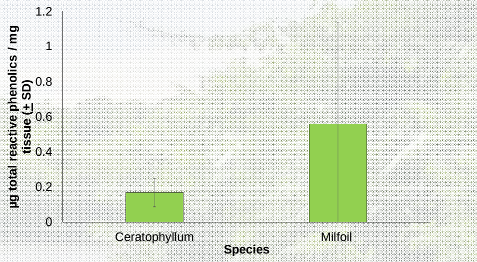


Figure 5 (left). Mean mg condensed tannins per g tissue (+SD) in invasive *Myriophyllum spicatum* (milfoil) and native *Ceratophyllum demersum.* Surprisingly, the native plant produced significantly more condensed tannins than did the invasive species (t-test: $t_{14}=4.66$, $p<0.001$, df=14, n=5).

Figure 6 (right). Mean µg total reactive phenolics per mg tissue (+SD) in invasive *Myriophyllum spicatum* (milfoil) and native *Ceratophyllum demersum*. Invasive milfoil produced more than double the total reactive phenolics than the native plant, though the difference was only marginally significant, likely due to high variation between milfoil samples (t-test: $t_9=2.118$, $p=0.063$, n=5).



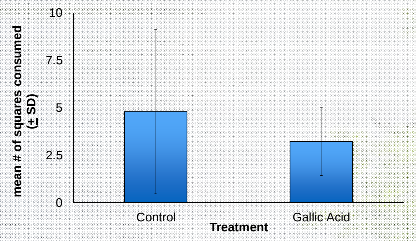


Figure 7 (left). Mean number of food squares (+ SD) consumed by amphipods (*Hyalella azteca*) during choice feeding trials comparing consumption of palatable control food and food containing 0.1% gallic acid. Amphipods consumed similar amounts of each treatment (paired t-test: $t_8=1.00$, $p=0.347$, n=10).

Conclusions

- Contrary to our expectations, native *Ceratophyllum demersum* contained higher condensed tannin levels than invasive milfoil (Fig. 5), while invasive milfoil produced more of the smaller phenolic compounds than *C. demersum* did (Fig. 6), as we hypothesized.
- Amphipods (*Hyalella azteca*) showed no preference for the control food, as there was no significant difference in consumption of the control vs. the gallic acid treated diets (Fig. 7). It is possible that compounds other than gallic acid affect herbivore food choices, so future studies should test feeding preferences with additional compounds.
- Based on these results, we cannot confidently conclude whether impact the interactions between *M. spicatum* and *C. demersum.*
- Overall, our results suggests that if phenolics do indeed play a role in milfoil invasion success, it is most likely due to the presence of small phenolic compounds rather than large phenolics like condensed tannins.

Literature Cited

1. Callaway RM and Ridenour WM. 2004. Novel weapons: invasive success and the evolution of increased competitive ability. Frontiers in Ecology and the Environment 2: 436-443
2. Constabel CP. 1999. A survey of herbivore – inducible defensive proteins and phytochemicals. In: AA Agrawal, S Tuzun, and E Bent (eds). Induced Plant Defenses Against Pathogens and Herbivores. APS Press, St. Paul, MN pp137-166

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