Patterns of chemical defense production and amphipod grazing in invasive *Myriophyllum spicatum* and native *Ceratophyllum demersum*

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**Introduction**

Submerged aquatic plants provide many ecosystem services, such as protecting against erosion, water retention, nutrient cycling, and providing habitat. *Myriophyllum spicatum* (hereafter known as milfoil) readily colonizes new habitats and can outcompete and replace native vegetation, which threatens those services. Invasive species can have a variety of negative impacts on aquatic communities; however, the mechanism leading to successful plant invasions remains unclear. Milfoil produces high levels of chemically deterrent phenolics, especially in its apical tissues, that make it unpleasant to some aquatic herbivores. If invasive species produce higher levels of chemical deterrents than native plants do, then native species may experience greater tissue loss due to herbivory than more chemically defended invasive plants. Both chemical deterrents and herbivory are unlikely to be uniformly distributed within invasive and native plant tissues, but that has not been fully explored.

This study investigated the distribution of phenolics in invasive *Myriophyllum spicatum* (milfoil) and native *Ceratophyllum demersum* (coontail) and the effects of phenolics on amphipod grazing.

**Hypotheses**

1. Aquatic plants defend their most valuable tissue (stem apex - required for regrowth) more than less valuable tissue (middle portion of stem), resulting in higher levels of chemical deterrent (phenolics) in apical tissues than in middle tissues.
2. High levels of chemical deterrent (phenolics) reduce herbivory, leading amphipods to prefer feeding on low-phenolic, native *Ceratophyllum demersum* compared to high-phenolic, invasive *Myriophyllum spicatum*.

**Methods**

**Plant Collection & Preparation**

Native coontail, invasive milfoil, and amphipods were collected from Osbourne Pond in Derby, CT for use in lab experimentation.

- Plants were held in 7-L tanks containing bubbled tap water, placed by the window to receive natural sunlight.
- 10 ml tubes were filled with either apical or middle portions of either milfoil or coontail and flash frozen at -80 degrees, then freeze dried and ground for phenolic analysis and use in feeding trials with artificial diets.

**Phenolic Analysis**

- 1 mL acetic acid in 70% acetone was used to extract phenolics from freeze-dried, ground milfoil and coontail samples.
- Total reactive phenolics were measured using the colorimetric Folin-Denis assay.

**Live-Plant Feeding Experiment**

- Live plant material from milfoil and coontail apex and mid stems were cut into 2-cm fragments, which were weighed before being placed into 500-ml bowls containing ~250 ml spring water.
- Amphipods were added to each bowl (10 amphipods per bowl, n = 5 of four treatments: milfoil apex, milfoil mid, coontail apex, coontail mid).
- Five autogenic control replicates of each treatment with live plant material but no amphipods were included to account for plant growth.
- After one week, plant tissue was removed from each bowl, blotted to remove excess water, and weighed to calculate the change in weight (g).
- Average growth in controls was subtracted from the experiment changes in weight to calculate a corrected change in weight.

**Artificial Diet Experiment**

- Artificial diets of 0.3 g agar, 20 ml deionized water, and 0.5 g of milfoil or coontail tissue from either the apex or the middle stem were spread across fiberglass screen (16 squares x 16 squares of 1-mm mesh) and allowed to solidify.
- Amphipods were added to 500-ml bowls containing ~250 ml spring water containing one piece of artificial food (10 amphipods per bowl, n = 5 of four treatments: milfoil apex, milfoil mid, coontail apex, coontail mid).
- Five autogenic control replicates of each diet treatment without amphipods were included to account for agar loss.
- The number of squares cleared of food was recorded each day for four days.

**Results**

**Figure 1.** Mean mg phenolics/g of plant tissue (+ SD) in apical and middle invasive *Myriophyllum spicatum* (milfoil) and native *Ceratophyllum demersum* (coontail) tissues. Milfoil apex tissue contained significantly more phenolics than milfoil mid tissue or either tissue from coontail (Species: F1,26 = 111.380, p < 0.001; Tissue: F1,1 = 7.140, p = 0.011; Species*Tissue: F1,26 = 12.084, p = 0.001).

**Figure 2.** Mean corrected change in live plant apical or middle tissue weight (g ± SD) from invasive *Myriophyllum spicatum* (milfoil) and native *Ceratophyllum demersum* (coontail) exposed to amphipod grazing for one week. Milfoil mid tissue grew significantly more than milfoil apex tissue, but there was no difference in growth between species (Species: F1,16 = 0.681, p = 0.422; Tissue: F1,16 = 8.775, p = 0.009; Species*Tissue: F1,16 = 6.767, p = 0.019).

**Figure 3.** Mean number of artificial diet squares made with tissue from milfoil apex, milfoil mid, coontail apex, and coontail mid consumed by amphipods after four days. There were no significant differences in the number of squares consumed among treatments (Species: F1,9 = 0.621, p = 0.442; Tissue: F1,9 = 0.033, p = 0.858; Species*Tissue: F1,9 = 0.621, p = 0.442).

**Literature Cited**


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Conclusions

As predicted, apical tissue from invasive *M. spicatum* contained more phenolics than its middle section or either tissue type from native *C. demersum* (Fig. 1). Although invasive milfoil allocated more phenolic content to its apical meristem than to its (presumably less valuable) middle tissues, native coontail did not defend its apical tissues with phenolics more than its middle tissues (Fig. 1).

In the live plant feeding experiment, amphipods consumed more milfoil apex tissue than middle tissue but consumed similar amounts of milfoil apex, coontail mid, and coontail apex tissues (Fig. 2). There was little overall consumption of the artificial diet, so we cannot draw any conclusions from those data (Fig. 3).

Overall, our results demonstrate that high phenolic content in the milfoil apex did not inhibit amphipod feeding. However, amphipods did avoid consuming milfoil’s middle tissues (Fig. 3), suggesting that other defenses may be present in those tissues.

The high levels of phenolics we found in milfoil’s apical tissue, suggesting that other defenses may be present in those tissues. Future work will explore this possibility.