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# Visual Plant Differentiation by the Milfoil Weevil, *Euhrychiopsis lecontei* Dietz (Coleoptera: Curculionidae)

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## Introduction

*Euhrychiopsis lecontei* Dietz (Coleoptera: Curculionidae) is an aquatic weevil native to the northern United States that specializes on plants in the genus *Myriophyllum* (milfoils). *Euhrychiopsis lecontei* is currently being used in the U.S. as a biological control agent for the invasive Eurasian watermilfoil (*Myriophyllum spicatum* L.: Haloragaceae; Newman 2004). The weevil has expanded its host range to include *M. spicatum* since the plant's introduction from Eurasia in the 1940s. Field and laboratory studies have shown that *E. lecontei* reduces *M. spicatum* densities, and also prefers *M. spicatum* over other (native) milfoils (see Newman 2004 for summary of *E. lecontei* life history and use as a biological control agent).

*Euhrychiopsis lecontei* can use visual cues for plant location (Reeves et al. 2009). However, during no-choice plant differentiation trials, weevils were attracted to both *M. spicatum* and coontail (*Ceratophyllum demersum* L.: Ceratophyllaceae; Reeves et al. 2009). Specifically, weevils swam equally quickly to sealed vials containing one or the other of these plants when one individual plant stem was the only visual stimulus in the behavioral arenas used. We present results here describing how the weevils responded to choice trials where both *M. spicatum* and *C. demersum* were placed side by side in the behavioral arenas. This short communication expands on the work presented by Reeves et al. (2009) which showed that weevils can use vision for at least initial host-plant detection, but may not be able to visually differentiate between plant species. Because Reeves et al. (2009) did not conclusively show in no-choice trials that weevils were unable to visually

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differentiate plants, the goal of this study was to more closely examine the question of visual plant differentiation by *E. lecontei*.

## Methods

The same weevil husbandry methods, behavioral arenas, and basic experimental methodology were used as in Reeves et al. (2009). Plant stems (*M. spicatum* and *C. demersum*; meristems used for all experiments) were sealed in water-filled, one dram, clear glass vials (dimensions: ~4.5 cm tall; ~1.5 cm diameter) to prevent chemical cues from altering weevil behavior. Two vials (one vial containing *M. spicatum*; one vial containing *C. demersum*; plant stems filled length of vial) were horizontally placed side by side (vial lids pointing away from one another) in random locations at the edges of the circular arenas (17 cm diameter) in ~2 cm deep water. The left to right order of the plant species was randomized. Vials were placed side by side (as opposed to opposite one another) in the arenas to prevent the weevils from swimming to the first vial to enter their field of view (i.e., the vial they were facing when released into the arena) since the weevils are attracted to both plant species when viewed singularly (Reeves et al. 2009). Weevils were individually released into the center of the arena and the vial they contacted first was recorded. These trials were replicated with 32 weevils (weevil age unknown for all experiments; weevils for all experiments reared on *M. spicatum*), both male and female.

To examine the possibility that weevils are using plant shape or growth form over plant color to differentiate host-plants (coontail is a similar color green), choice and no-choice trials were performed using sealed brown (i.e., yellow-brown, dead/ decomposing stems) vs. green (healthy) *M. spicatum* stems with similar growth forms. The brown and green colors, along with similarity in growth form (i.e., stem size and leaf density), were visually determined subjectively by the experimenter. For the no-choice trials, the weevils ($n$=20) were individually released into the center of an arena and the time to vial contact was recorded for brown vs. green stems separately, as in the no-choice *M. spicatum* vs. *C. demersum* trials in Reeves et al. (2009). Next, choice trials were performed by placing sealed brown and green stems side by side (in random left to right order) in the arenas (as in the plant species choice trials above), and recording which stem the weevils ($n$=32) contacted first. The smaller sample size for the no-choice color trials resulted from lower weevil availability for that experiment and no clear indication that more weevils would have affected the outcome of the experiment.

For all experiments, lame weevils that could not move easily throughout the behavioral arenas or those that appeared to swim randomly with no interest in the plant samples were excluded from the experiments and analyses. Seven weevils were excluded from the milfoil vs. coontail choice experiment, nine weevils were excluded from the no-choice brown vs. green milfoil experiment, and three weevils were excluded from the brown vs. green milfoil choice experiment. All sample sizes presented in the Methods and Results and Discussion sections are responsive weevils and do not include the lame weevils noted here. The trials for all experiments were designed to be 10 min long, however most weevils made clear choices and swam to the plant stems within 1 min. Most weevils would swim directly toward the plant stems when they entered their field of view and then swim against the vial in which the plant stem was sealed for several seconds seemingly to get to the plant.

## Results and Discussion

When the weevils ($n$=32) were given a choice between *M. spicatum* and *C. demersum* sealed in vials, they showed a visual preference for *M. spicatum* over *C. demersum* (22 chose *M. spicatum*; 10 chose *C. demersum*; $G$-test of goodness-of-fit $G$=4.6120; $P$=0.0318). The results of this experiment suggest that *E. lecontei* may be able to visually differentiate between aquatic plant species while under water, and contradicts the initial findings of the no-choice tests between *M. spicatum* and *C. demersum* in Reeves et al. (2009) where the weevils were attracted to both plants when time to plant contact was measured instead of direct plant choice/preference. The negative results of this initial experiment reported in Reeves et al. (2009) were likely due to the fact that the weevils are poor swimmers, and may have recognized *C. demersum* as a plant and therefore safety when it was the only visual stimulus present in the no-choice trials. However, when given a choice here, the weevils visually preferred *M. spicatum* to *C. demersum*. In the case of *E. lecontei*, and likely other systems, both choice and no-choice trials can be useful (but may produce different results) when quantifying host-plant location/selection behavior. That is, Reeves et al. (2009) showed in no-choice trials that *E. lecontei* is potentially visually attracted to plants in general (likely for safety), but here it is shown that weevils can visually select the appropriate host when given a choice between a host and non-host species. Both of these results stem from methodological differences between Reeves et al. (2009) and the work presented here, and both give useful indications about the plant locating mechanisms of *E. lecontei*.

In the no-choice trials between brown and green stems, weevils were equally quickly attracted to brown and green stems when they were individually placed in the arenas. Nine of the twenty responsive weevils (45%) swam to the brown plant faster, and the remaining 11 responsive weevils (55%) swam to the green plant faster. There were no differences in the average time to vial contact for brown plants (11.9±7.5 sec) and the average to green plants (10.8±6.9 sec) (paired $t$-test $t$=−0.8223; $P$=0.4211). When choice trials were performed with brown and green stems placed side-by-side ($n$=32 weevils), similar results were obtained: 15 responsive weevils contacted the brown plant first, whereas 17 responsive weevils contacted the green plant first ($G$-test of goodness-of-fit $G$=0.1251; $P$=0.7200).

Based on the data presented here, it seems as though *E. lecontei* can visually differentiate between plant species, at least when given a choice between *M. spicatum* and *C. demersum*. These data expand on those presented in Reeves et al. (2009) by demonstrating that vision is important for host-plant detection (and potentially selection) by *E. lecontei*. Prokopy and Owens (1978) speculated that monophagous/oligophagous insects may be more visually acute in host-plant detection than polyphagous insects. Our data support this notion, as *E. lecontei*, a weevil specializing on watermilfoils, is visually more attracted to its host-plant than a non-host-plant species.

The leaves of two plants used here have different growth forms. *Myriophyllum spicatum* has whorled compound leaves with many leaflets, and *C. demersum* has stiff, whorled, single needle-like leaves, so it is possible that the weevils are discerning plant species based on their shape, especially given that weevils do not appear to distinguish between brown and green stems with similar growth form in either choice or no-choice trials. Future research should focus on determining the extent to which plant color or plant form is used by *E. lecontei* to visually locate potential host-plants, and which colors or shapes are most attractive to the weevils. This research should clearly include the manipulation of both plant shape and color. Also, it would be useful to examine weevil response to more plant species, including native milfoils (e.g., *Myriophyllum sibiricum* Komarov) that have similar leaf shapes and colors to *M. spicatum*.

Gaining an understanding of host-finding behavior in this and other biological control systems may eventually allow us to better predict when and where biological control agents can find and damage their target host-plants. In fact, mechanisms of host location are among the most important factors listed by Cuda et al. (2008) for understanding and predicting efficacy of biological control agents of submersed aquatic weeds. Beyond efficacy prediction, a deeper understanding of the role of vision in host-finding behavior also may help lead to the development of traps to assess weevil presence and abundance. Because trap color and placement (Hoback et al. 1999) and trap type (Bloem et al. 2002) can influence the outcome of insect surveys, a greater understanding of the specific visual stimuli that are attractive to *E. lecontei* is essential for developing efficient traps for this weevil. The work presented here and by Reeves et al. (2009) advances our understanding of host-locating mechanisms used by *E. lecontei* and thus may eventually lead to better prediction of biological control efficacy and trap development.

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