A Brine Shrimp Bioassay Comparing Anti-herbivory Compounds in the Endangered Wildflower *Senna hebecarpa* and Four Other Temperate Wildflowers

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Background and Objectives

Northern Wild Senna (*Senna hebecarpa*) is an endangered wildflower that was once widespread throughout much of New England. There have been several hypothesized causes to the species decline, but one factor that does not appear to be contributing is damage from herbivory. We have found little evidence of herbivory within a wild population at our field site in Amherst, NH; an observation that is consistent with other reports of cows "shunning" the plant in pastures (Clark 2001). Anti-herbivory compounds have not been extracted and identified for *S. hebecarpa*, but several other *Senna* species are know to be purgatives or mild laxatives due to the presence of cathartic acid (Clark 2001). The objectives of this study were to:

1.Test for the presence of phytotoxic compounds in leaves of *S. hebecarpa* using a brine shrimp bioassay

2. Compare the relative phytotoxicity of *S. hebecarpa* leaves to those of four other native wildflowers (Figure 1)

Methods

- Collected plant material from Saint Anselm College's monastery garden and adjacent natural areas 17 September 2009
- Stored leaf material in -80°C freezer
- Prepared leaf extract using mortar and pestle followed by soaking in 100% methanol
- Conducted brine shrimp (*Artemia salina*, Figure 2) bioassay as per treatments in Table 1 (n = 5 vials of 10 shrimp per treatment)
- Constructed dose response curves to calculate the extract concentration that kills 25% (LC_{25}) or 50% (LC_{50}) of 4d old shrimp within 24h and 48h, respectively (Figure 2)

Table 1. Calculations for final concentration of plant material for each treatment

Treatment	10 µg/ml Extract	µg/ml of Plant Material	Final Volume Seawater	Final Concentration Plant Material
Seawater control	0	0	5 ml	0
Methanol control	0, 50 µl methanol	0	5 ml	0
Low	5 µl	50 µg	5 ml	10 µg/ml
Medium	50 µl	500 µg	5 ml	100 µg/ml
High	500 µl	5000µg	5 ml	1000 µg/ml

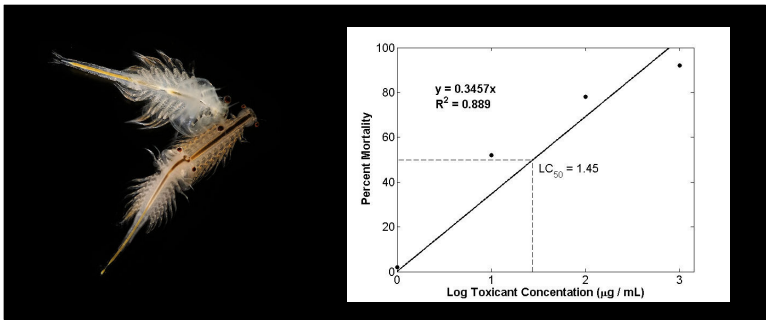

Figure 2. Linear regression plot of toxicant concentration versus shrimp mortality for *Solanum dulcamara*. Dashed lines indicate the lethal concentration of extract that kills 50% of shrimp (LC_{50}). Photo by Hans Hillewaert .

References

- Clark, F. H. 2001. *Senna hebecarpa* (Northern Wild Senna) Conservation and Research Plan. New England Plant Conservation Program, Framingham, Massachusetts, USA (http://www.newfs.org)

Figure 1. Study species from left to right: *A. syriaca, P. americana, P. pensylvanicum, S. hebecarpa, S. dulcamara*

Results and Conclusions

- Shrimp mortality rates for both the seawater and methanol control treatments (Table 1) were not statistically different from zero ($P > 0.05$)
- Significant levels of shrimp mortality exposed to leaf extracts confirm the presence of phytotoxins in *S. hebecarpa* leaf tissue (Tables 3, 4)
- *Senna hebecarpa*'s LC_{50} of 63 µg/ml places it within the same range of phytotoxicity (Table 2) as chemically well-defended nightshade (*S. dulcamara*) and milkweed (*A. syriaca*)

Table 2. Lethal concentration values (LC) at 24h and 48h. Reported values are in µg/ml calculated from log toxicant values using inverse log equation ($y = 10^x$).

Species	24 hr LC_{25} (µg/ml)	48 hr LC_{50} (µg/ml)
Asclepias syriaca	102	17
Phytolacca americana	871	100,000
Polygonum pensylvanicum	79	251
Senna hebecarpa	63	355
Solanum dulcamara	28	1,738

Tables 3 and 4. One-way ANOVA comparing brine shrimp mortality (mean ± S.E.) after 24h (top) and 48h (bottom) for five plant species exposed to different concentrations of plant leaf extract.

	Concentration of Leaf Extract (µg/ml)		
Species	**10**	**100**	**1000**
Asclepias syriaca	$0.38^{a} \pm 0.06$	$0.48^{a} \pm 0.03$	$0.50^{a} \pm 0.12$
Phytolacca americana	$0.04^{b} \pm 0.03$	$0.12^{b} \pm 0.05$	$0.14^{b} \pm 0.05$
Polygonum pensylvanicum	$0.04^{b} \pm 0.04$	$0.26^{b} \pm 0.05$	$0.30^{ab} \pm 0.05$
Senna hebecarpa	$0.02^{b} \pm 0.02$	-	$0.32^{ab} \pm 0.06$
Solanum dulcamara	$0.02^{b} \pm 0.02$	$0.14^{b} \pm 0.07$	$0.26^{ab} \pm 0.07$
ANOVA *F* ratio	19.2	9.9	5.1
P value	<0.005	<0.005	0.005

	Concentration of Leaf Extract (µg/ml)		
Species	**10**	**100**	**1000**
Asclepias syriaca	$0.50^{a} \pm 0.03$	$0.50^{ab} \pm 0.05$	$0.66^{a} \pm 0.05$
Phytolacca americana	$0.16^{b} \pm 0.05$	$0.36^{a} \pm 0.05$	$0.50^{a} \pm 0.07$
Polygonum pensylvanicum	-	$0.66^{ab} \pm 0.11$	$0.70^{a} \pm 0.06$
Senna hebecarpa	$0.02^{b} \pm 0.02$	-	$0.92^{b} \pm 0.04$
Solanum dulcamara	$0.52^{a} \pm 0.04$	$0.78^{b} \pm 0.06$	$0.92^{b} \pm 0.04$
ANOVA *F* ratio	46.0	6.9	12.2
P value	<0.005	<0.005	<0.005

Different letters denote significant differences ($p \leq 0.05$) between species within each extract concentration as tested by Tukey's post-hoc comparisons.

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