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PCCI research report
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Project Title: Effects of tree-of-heaven toxins on rodent herbivory.
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Introduction

The invasive tree species Tree-of-Heaven (TOH), *Ailanthus altissima*, is native to Asia and has been introduced to the United States multiple times over the past two centuries. Several aspects of TOH ecology facilitate its invasiveness, including early & prolific reproduction, wind dispersed seeds, aggressive clonal reproduction, and production of toxins that limit local plant growth and damage from many insect and rodent herbivores (Heisey, 1996; Ostfeld et al., 1997). TOH toxicity to plants varies seasonally (Voigt & Mergen, 1962), among tissue types (Heisey, 1990), and is presumed to vary among individuals (currently under investigation by Preston Aldrich – Benedictine University and Gary Greer – GVSU). TOH toxins discourage some, but not all, herbivores (Tsao et al., 2002; Ostfeld et al., 1997). The goal of our project was to determine if variation in TOH toxin levels affected rodent herbivory on known high quality foods.

Methods

We tested for variation in rodent response to food treated with different concentrations of TOH toxins in 5 forest and 5 meadow sites. Each experiment was replicated 6 times. Rabbit chow will be used in the first two experiments since it is known to be a preferred, high-quality food source for rodents (Batzli, 1985). Treated food was placed into (a) feeding stations to measure relative consumption rates and (b) live traps to determine rodent species involved. Our third experiment utilized sunflower seeds to measure relative consumption rates by granivorous rodents (i.e., seed-eating). TOH toxins were obtained via methanol extraction (Lin et al., 1995; De Feo et al., 2003). A constant volume of extracted liquid was applied to food in all experiments (i.e., 3 ml/20g food).

Experiment #1 – Two large feeding stations per site were placed in the field two days prior to food addition. Each feeding station contained all food treatments (n = 3 + 2 controls listed below), pre-weighed food was placed in Petri dishes arranged in random order at the center of the station (feeding stations measure ≈ 80 x 50 x 18 cm, see Figure 1). Two types of controls were used (1) food treated with distilled water and (2) food treated with an extract from the combination of northern red and white oak, sugar and red maple, and flowering dogwood applied at high concentration. Three levels of TOH toxins were applied to food: (3) low, (4) medium, and (5) high concentrations. These three treatments were equivalent to amount of TOH toxins found in 1g, 2.5g, and 5g of TOH tissue, respectively. Rodents such as meadow voles are known to consume ≈ 20g of food daily (Caron et al., 1985). We purposefully chose these relatively modest treatment levels to reflect biologically relevant values of toxins that small mammals might typically encounter while foraging. Food was collected, dried, and weighed to estimate food consumption every two days over a ten day period.

Experiment #2 – Live trapping trials utilized traps pre-baited and baited four treatment combinations were used to determine species-specific responses to different TOH concentrations. Ten trapping stations were established at each site. Each trapping station consisted of two live traps placed in close proximity (≈ 25 cm, see Figure 1). One trap received bait treated with TOH toxins (i.e., low or high concentration) the second trap received control bait (one of the two controls described for experiment #1). Live traps were pre-baited for two days with the appropriate food treatment. Bait was replaced every two days. Traps were set in the evening for ten consecutive nights. Traps were checked and closed the following morning. Captured individuals were identified to species, marked, and standard trapping data was collected. Traps were cleaned after captures occurred. All traps were cleaned between subsequent trials.

Experiment #3 – Five small feeding stations per site were placed in the field two days prior to food addition, one of each treatment listed in experiment #1 (feeding stations measure ≈ 15 x 15 x 5 cm, see Figure 1). Each station was filled with sand (≈ 500ml) and 50 treated sunflower seeds, stations were placed in the ground so that the top of the container was level with the ground. Plywood cover boards (25 x 25 cm) were placed over feeding stations to discourage foraging from larger animals and to keep the sand dry. Seeds were collected and replaced every two days over a ten day period (i.e., giving-up density – a commonly used method for estimating risk sensitive foraging, e.g., Orrock et al., 2004).

We used general linear models to analyze our data (Statistica software: StatSoft, Inc., 2002). Data not conforming to the assumptions of normality were transformed. Vertical bars in figures denote 95% confidence intervals.

Figure 1. Feeding stations and live trap stations used in our three experiments.

Results

Experiment #1 – Large feeding stations

Food consumption was significantly impacted by time ($F_{5,540}=9.42$, $P<0.0001$), habitat ($F_{1,540}=424.12$, $P<0.0001$), and their interaction ($F_{5,540}=21.22$, $P<0.0001$). The main effect of treatment had no effect on food consumption ($F_{4,540}=0.18$, $P=0.95$). Food consumption was higher in meadow than forest and generally declined in meadow while consumption increased to a plateau in forest (Figure 2).

Experiment #2 – Live trapping

Total live trapping effort consisted of 12,000 trap nights (20 traps/site * 10 sites * 10 nights/replicate * 6 replicates). Eight species were captured 274 times, resulting in 2.3% trapping efficiency (Table 1). Captures in forest sites were dominated by white-footed mice, *Peromyscus leucopus* (93%). Captures were much less frequent in meadows than forests (Table 2). Meadow voles, *Microtus pennsylvanicus*, and meadow jumping mice, *Zapus hudsonius*, were most commonly captured in meadow sites (39% & 35%, respectively). Time ($F_{5,552}=0.30$, $P=0.88$), habitat ($F_{1,552}=0.00$, $P=1.00$), and treatment ($F_{3,552}=1.70$, $P=0.16$) had no affect on captures. No significant difference was noted in the number of captures between control and TOH treatments (Figure 3).

Experiment #3 – Small feeding stations

Seed consumption was significantly impacted by time ($F_{5,240}=6.21$, $P<0.0001$) and habitat ($F_{1,240}=142.30$, $P<0.0001$). The main effect of treatment had no effect on seed consumption ($F_{4,240}=0.77$, $P=0.54$). Food consumption was higher in forest than meadow and fluctuated in both habitats throughout the study (Figure 4).

Table 2. Summary of the 274 livetrap captures in forest and meadow sites.

Forest		Meadow	
Species	Captures	Species	Captures
White-footed Mouse	226	Meadow Vole	12
E. Chipmunk	8	Meadow Jumping Mouse	11
N. Short-tailed Shrew	6	E. Chipmunk	4
Flying Squirrel	3	E. Cottontail	3
		Oppossum	1
Total captures	**243**		**31**

Discussion

In all three experiments no main effect of food treatment was observed. We interpret this lack of discrimination among treatments to indicate the range of TOH toxins we tested were below some threshold level that had been shown to inhibit foraging in previous studies (e.g., Ostfeld et al., 1997). Ostfeld et al. (1997) found that white-footed mice avoided consuming TOH seeds when other seeds were available. In contrast, meadow voles would readily consume TOH seedlings. We await our colleague's findings on the natural range of variation of toxins found in TOH tissue and among geographic localities to direct our future efforts. It has already been established that other herbivores are impacted by genetic differences among individual plants, presumably mediated through levels of defensive compounds (e.g., beaver – Baily et al., 2004; insects – Prittinen et al., 2003).

Time and habitat effects were found in experiments #1 & #3. Consumption of rabbit chow in experiment #1 was higher in meadow than forest habitats. This pattern was reversed in experiment #3 which utilized sunflower seeds. These patterns of consumption are readily explained by the dietary preferences of the small mammals involved in this study. Meadow voles consume green plant material and prefer high quality foods such as rabbit chow (Batzli, 1985), whereas other local rodents generally consume seeds (e.g., white-footed mice). We have no clear explanation for the time effects observed in this study (see Figures 2 & 4).

Disturbance levels were relatively high, especially for experiment #1. Larger mammals were also foraging from our feeding stations despite our efforts to prevent such occurrences. Raccoons, *Procyon lotor*, eastern cottontails, *Sylvilagus floridanus*, and white-tailed deer, *Odocoileus virginianus*, were the main non-target species involved in these disturbances. Analyses excluding disturbed feeding stations yielded similar results to those presented.

Conclusions

Low concentrations of TOH toxins do not inhibit rodent herbivory. More research is needed to determine the range of TOH toxin levels in nature. The growing presence of TOH in eastern deciduous forests is a growing conservation concern (Lawrence et al., 1991; Call & Nilsen, 2003). A better understanding of factors impacting TOH seed and seedling predation will help forest managers to better combat this invasive species.

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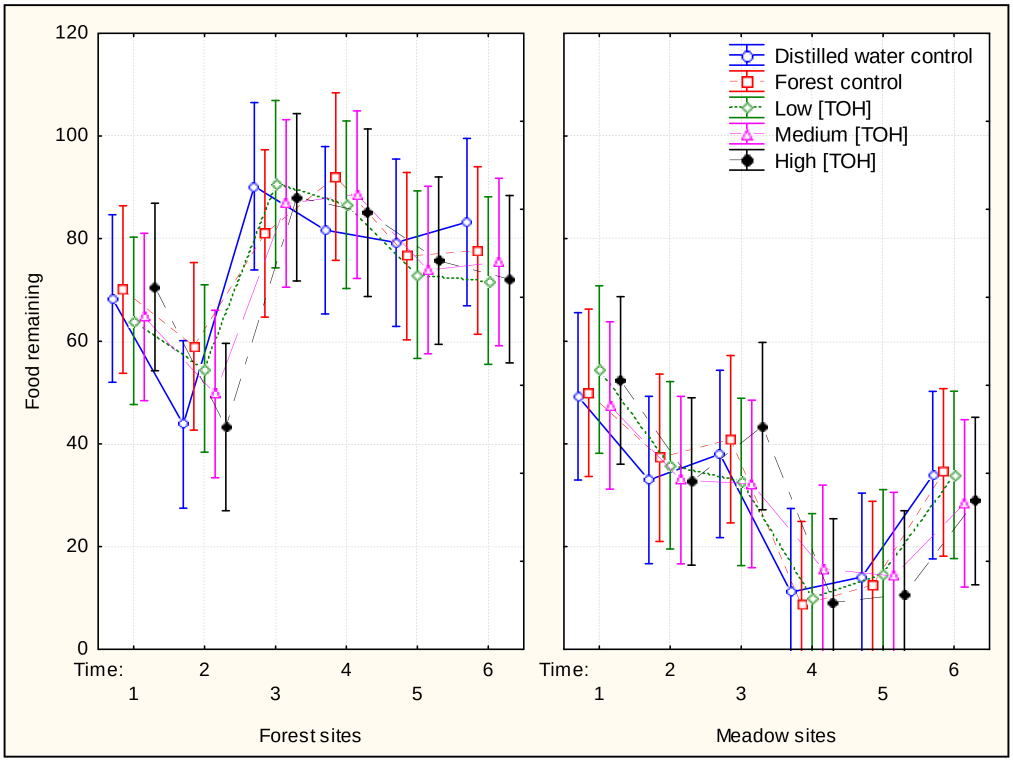

Figure 2. Cumulative amounts of food remaining in large feeding stations. Values shown are means for the five forest and five meadow sites. Time refers to each replicate of the experiment.

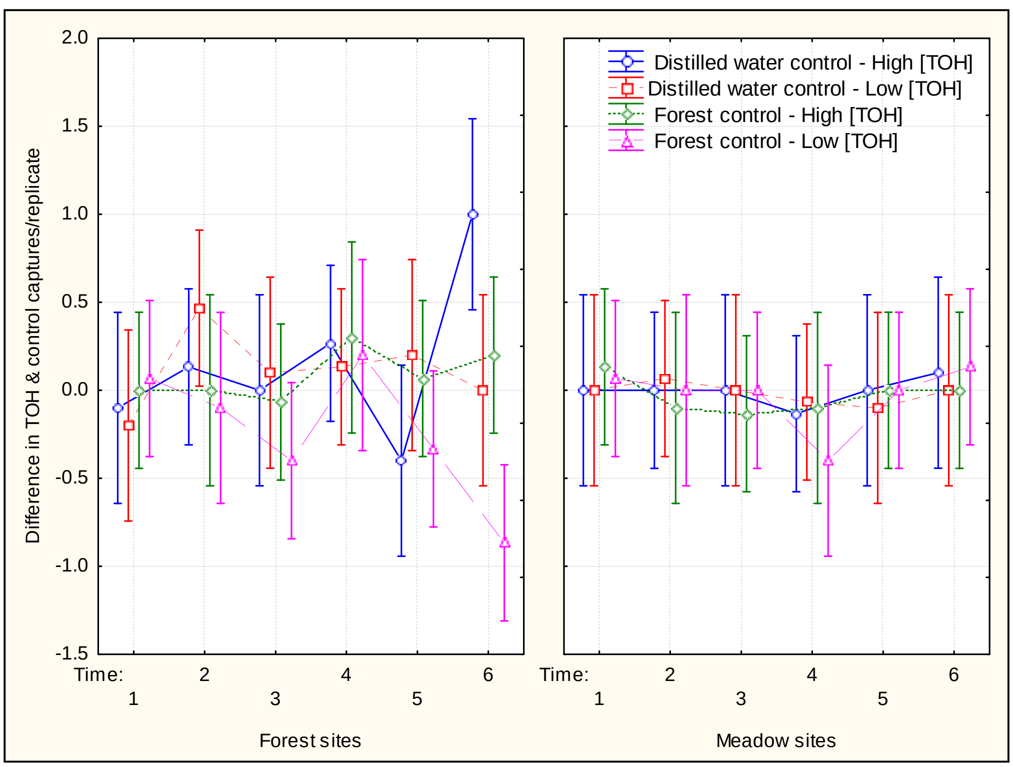

Figure 3. Differences in capture numbers between control and TOH treated live traps. Values shown are means for the five forest and five meadow sites. Time refers to each replicate of the experiment.

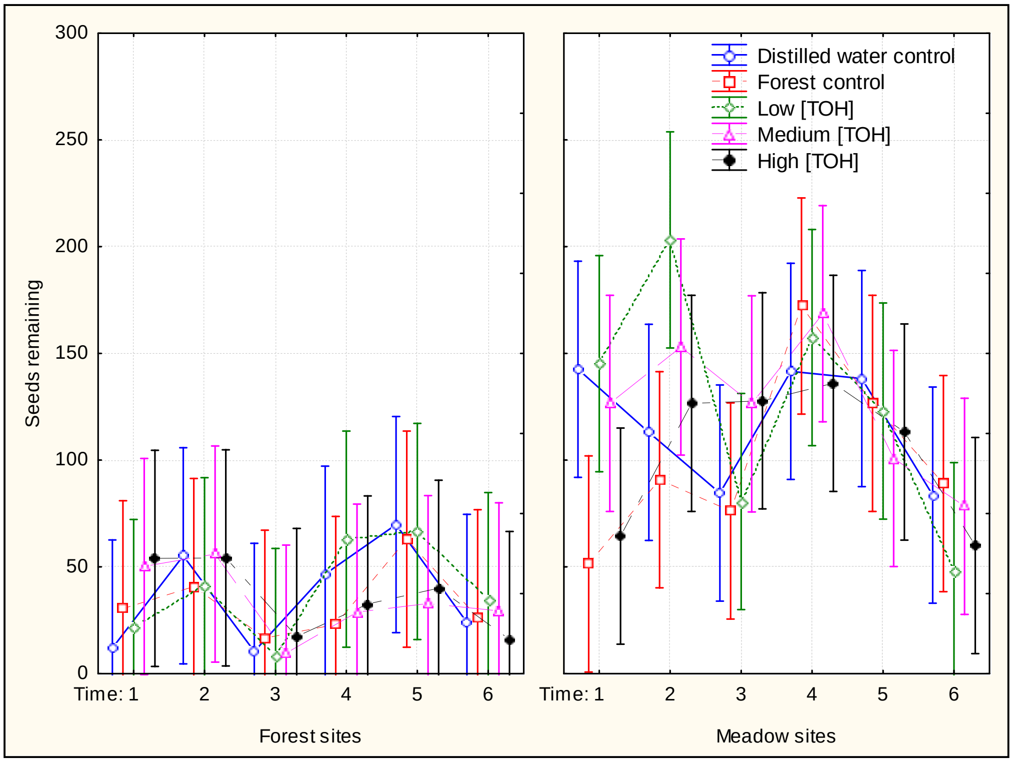

Figure 4. Cumulative amounts of seeds remaining in small feeding stations. Values shown are means for the five forest and five meadow sites. Time refers to each replicate of the experiment.