Reproductive success of *Nicrophorus marginatus* in response to a broadly used invasive species herbicide
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Presented by:
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Abstract

Broadcast application of commercially available pesticides and herbicides can have unintended detrimental effects across trophic levels in ecosystems. In particular, some herbicides have been shown to negatively affect reproduction in certain invertebrates, even though the intended targets are undesired primary producers. One widely applied herbicide used to target the grasslands invasive forb *Sericea lespedeza* Cuneata is Remedy® Ultra (Dow Agrosciences, active ingredient, Triclopyr). Although Remedy® Ultra has been shown to have low toxicity, its effects on animal behavior or reproduction has not been widely studied. Given its widespread use in grassland ecosystems, we asked whether application of the herbicide affects reproduction in, *Nicrophorus marginatus*, a native burying beetle found in grasslands that provides intensive biparental care of young in a breeding chamber beneath the soil. We predicted that if the chemical affects reproductive behavior and fitness of breeding *N. marginatus* adults then the frequency of carcass burial would be reduced, parental care behaviors would be influenced, and the number of offspring would differ from control. We used a combination of breeding experiments designed to replicate field application concentrations of Remedy® Ultra, and we measured carcass burial latency, number of offspring eclosed, individual size of offspring, and time to offspring emergence (latency). Overall, we found no significant difference in carcass burial latency, number of offspring, or offspring size between control and Remedy® Ultra treatment breedings. We did find, however, that offspring from Remedy® Ultra treatments emerged from the pupal stage significantly earlier than control offspring. Further, when F1 Remedy® Ultra offspring were bred for F2 offspring, they exhibited a significantly longer carcass burial latency. The increased rate of development (F1 and F2 generations) and increased time for carcass processing (F2) may indicate an effect of the growth hormone components of the herbicide on development. Future work will focus on neuroethological investigations designed to deduce the mechanisms tied to the herbicide’s effects on behavior and reproduction in beneficial invertebrates.
• What affects number and size of offspring in a brood?
  1. size of carrion (Bartlett, 1987)
  2. Parental investment (Scott & Traniello, 1990)

• Human influence on environment: Chemicals/herbicide
  • Invertebrates in grassland soil (Fox, 1964)
  • Frogs (Sower, Reed & Babbitt, 2000)
Remedy® Ultra

- Remedy® Ultra: triclopyr-based herbicide
- Effectively kills *Sericea lespedeza*
  (Tallgrass Legacy Alliance, 2009)
- Introduction: 1896; wide use: 1940s (Gucker, 2010)
- Invasive weed of Tallgrass Prairie Preserve in Oklahoma (20 yr seed dormancy!)

Red dot indicates location of Tallgrass Prairie Preserve (Osage County, OK)

*Sericea lespedeza*
Triclopyr Mode of Action

- Mimics a growth plant hormone (auxin)
- Causes rapid growth of plant cells
  (AgChemAccess2013, Ganapathy, 1997)

- Low soil persistence (microbial metabolism)
  - Photo degradation in water (Tatum, 2004)
- Low toxicity on animals except high doses
  (Ganapathy, 1997, Tsuda et al. 1987)
Primary Question

How will the reproductive success of *Nicrophorus marginatus* be affected by application Remedy® Ultra herbicide?
Hypotheses

• Remedy® Ultra will affect the reproductive behavior of male and female *Nicrophorus marginatus*.

• Remedy® Ultra will affect growth and development of *Nicrophorus marginatus*. 
Predictions

• If R®U does affect reproductive behavior, then...
  • burial latency of *N. marginatus* will be significantly greater for carrion covered in R®U herbicide.

• If R®U does affect growth and development, then...
  • When *Nicrophorus marginatus* offspring are raised on carrion sprayed with R®U, fewer offspring will emerge and fitness of offspring will decrease.

  • *Nicrophorus marginatus* larvae raised on R®U carrion will result in offspring with smaller average size than offspring emerging from control larvae.
Methods

• 40 containers of native OK soil
  • 20 control
  • 20 R®U sprayed on mouse carcass and soil at application level

• **Images** taken by web camera using Logitech programming

• **Burial latency**: checked every 4 hours

• Watered once every two days
Methods

• **Size:** measured pronotal width (PW) of parents and offspring using Image J

• **Documented:**
  • Burial latency
  • Number in brood
  • Size of parents and offspring

• **Data analysis tools:**
  • Shapiro-Wilk test for normality
  • Levene’s Test for Equality of Variances (t-tests)
  • Equal Variance Independent Samples T-Tests (round 1)
  • ANOVA (round 2)
# Results

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Remedy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Offspring</td>
<td>6.210 ± 7.443</td>
<td>7.150 ± 6.854</td>
</tr>
<tr>
<td>Offspring Size (mm)</td>
<td>6.989 ± 0.944</td>
<td>6.908 ± 0.586</td>
</tr>
<tr>
<td>Emergence (days)</td>
<td>57.578 ± 2.410</td>
<td>54.094 ± 2.757</td>
</tr>
</tbody>
</table>
Results

1. Burial Latency
   - No difference ($t=0.721$, $df=40$, $p=0.475$)
   - SPSS: Equal variance independent samples t-test
Results

• 2. Number of offspring
  • No difference \( t=-0.410, \text{ df}=40, \text{ p}=0.684 \)
  • SPSS: Equal variance independent samples \( t \)-test
Results

• 3. Size of Offspring (Pronotal Width)
  • No difference ($t= 0.013$, $DF=16.09$, $p=0.505$)
  • SPSS: Equal variance independent samples t-test
• 4. Emergence Latency (days)
  • Significant difference (t=3.212, df=22, p=0.004)
  • SPSS: Equal variance independent samples t-test
## Results

**Fitness of Remedy-treated offspring?**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Remedy</th>
<th>F1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Offspring Size (mm)</td>
<td>6.989 ± 0.944</td>
<td>6.908 ± 0.586</td>
<td>6.930 ± 0.710</td>
</tr>
<tr>
<td>Emergence (days)</td>
<td>57.578 ± 2.410</td>
<td>54.094 ± 2.757</td>
<td>58.240 ± 3.260</td>
</tr>
</tbody>
</table>
Results

1. Burial Latency
   - Significant difference ($f=10.883$, $df=2$, $P=0.001$)
Results

2. Number of offspring
   - No difference ($f = 0.717$, $df = 2$, $P = 0.493$)
Results

3. Size of Offspring (Pronotal Width)
   - No difference ($f = 0.036$, $df = 2$, $P = 0.965$)
Results

4. Emergence Latency (days)
   - Significant difference ($f = 0.163$, $df = 2$, $P = 0.001$)
Discussion

• **Accept Hypotheses:**
  • Increased burial latency for F1 generation
  • Reject hypothesis of lower fitness and smaller size

• **Emergence Latency:**
  • developmental effect?
    - Endocrine-disruptive chemicals (EDCs) and invertebrates (Thomas 2014)
    - Parental regurgitation=ingestion of herbicide
Future Research

1. Replicate Experiments with congeners, including *N. americanus*

2. How does removal of *Sericea lespedeza* or other invasive plants affect small animals/other food sources of burying beetles?
   - Research in natural habitat of Tallgrass Prairie Preserve in OK
   - Implications of research in captivity vs. natural habitat
Acknowledgements

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