Molecular Evidence for the Consumption of *Plutella xylostella* (Diamondback Moth) in Bat Guano

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United Tribes Technical College, USDA-NIFA BATS II (Award # 2015-38424-22669)
This is much better. It grabs my eye and makes me smile. I also can't wait to watch the rest of your presentation. Reminder to put in the rest of the bats grant award information. I sent it to you.
The Diamondback Moth (*Plutella xylostella*)

- Damage estimated at $1 billion annually
- The eggs are oval and flattened, measuring 0.44 mm long and 0.26 mm wide
- The yellowish pupae are about 8 mm long and are wrapped in a loose silk cocoon.
- The adult moth is approximately 8 to 9 mm long with a wing span of 12 to 15 mm
This includes other plants like...

Mandy Guinn, 7/28/2015

Sorry you probably don't need to put the plants just be ready to say them on this slide.

Mandy Guinn, 7/28/2015
Plutella xylostella Continued…..

- One of the most studied insects in the world
- Negative impacts
- Highly migratory
- Resistant to all major pesticides
- Rainfall and wind are known deterrents
Ecological Importance of Bats

- Help control insect/pest populations
- Pollinate plants
- Reseed deforested land
- Fertilizer
- Helpful in improving antibiotics
One thing that keeps catching my eye is that the titles and text boxes all vary in size and causes me to notice the variability. Try and keep them consistent.

Mandy Guinn, 7/28/2015
Bat Background

- Bats navigate by using sound waves
- Bats eat insects by mouth and sometimes capture by wing
- Bats are mammals
- Bats can be found almost everywhere in the world
Canola in North Dakota

- Nations leading producer
- Crucifer plant
- Seeds harvested for oil
- Economically important

![Canola field with barn](image)

**Canola Area Planted – States and United States: 2013-2015**

<table>
<thead>
<tr>
<th>State</th>
<th>2013 (1,000 acres)</th>
<th>2014 (1,000 acres)</th>
<th>2015 (1,000 acres)</th>
<th>Percent of previous year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idaho</td>
<td>44.0</td>
<td>55.0</td>
<td>25.0</td>
<td>71</td>
</tr>
<tr>
<td>Minnesota</td>
<td>17.0</td>
<td>14.0</td>
<td>19.0</td>
<td>136</td>
</tr>
<tr>
<td>Montana</td>
<td>72.0</td>
<td>60.0</td>
<td>60.0</td>
<td>96</td>
</tr>
<tr>
<td>North Dakota</td>
<td>220.0</td>
<td>120.0</td>
<td>120.0</td>
<td>100</td>
</tr>
<tr>
<td>Oklahoma</td>
<td>290.0</td>
<td>270.0</td>
<td>140.0</td>
<td>54</td>
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<tr>
<td>Oregon</td>
<td>13.0</td>
<td>11.0</td>
<td>5.0</td>
<td>45</td>
</tr>
<tr>
<td>Washington</td>
<td>37.0</td>
<td>51.0</td>
<td>30.0</td>
<td>59</td>
</tr>
<tr>
<td>Other States</td>
<td>40.0</td>
<td>70.0</td>
<td>70.0</td>
<td>100</td>
</tr>
<tr>
<td>United States</td>
<td>1,340.0</td>
<td>1,714.0</td>
<td>1,064.0</td>
<td>91</td>
</tr>
</tbody>
</table>

1. Intended plantings in 2015 as indicated by reports from farmers.
2. Other States include Colorado and Kansas. The 2015 estimate is carried forward from 2014. Final 2015 estimate for Other States will be published in Acreage released, June 2016.
Be ready to explain this graph. I think you are but because it is on the slide you need to reference it.
Mandy Guinn, 7/28/2015
Purpose for research

- If the bats captured have been consuming *Plutella xylostella* then the evidence will be found within their guano through molecular analysis.
- Bats provide a vital ecological role
- Throughout the United States bats are worth more than $4 billion a year in reduced crop damage and pesticide use.
- Worth more than $23 billion worldwide
Need to have a purpose statement and research question. So we give the audience something to reference.

Mandy Guinn, 7/28/2015
Locations

- UTTC Campus
- UTTC Attics
- Cross Ranch
- Anamoose
- Painted Woods
Capturing and Collecting

- Mist nets
- Gloves
- Patterson Acoustic Detector
- Cloth bags
- Tweezers
- Rubbing alcohol
Health Analysis

**Epfu Health**

**Mylu Health**
DNA analysis

- 56 samples
- Prepped with ZR Fecal DNA microprep kit
Let's just keep this slide 100% DNA extraction. We can do PCR on next slide.

Mandy Guinn, 7/28/2015
• Optimized 14 samples on campus
• PCR > primers > nucleotides > Hot taq > DNA polymerase
• 1\textsuperscript{st} step: Denature
• 2\textsuperscript{nd} step: Annealing
• 3\textsuperscript{rd} step: Elongation
  • Cycling conditions
    1. 95C for 3 minutes
    2. 94C for 1 minutes
    3. 50C for 1 minutes
    4. 72C for 1.5 minutes
    5. 72C for 5 minutes
Primers used for PCR

- **NANCY**: CC CGGTAAAAATATATATAAAC TTC
- **K698**: TC AATTTATCGCTAAACTTCA GCC
- Target COI gene (mitochondrial)
- Higher success for digested insect sample
- COI is a key enzyme in aerobic metabolism (complex VI in electron transport chain)
- Approximately 600bp region was targeted
Add the full sequence
Functional Bioscience

- Sent in DNA
- First run unsuccessful changed annealing temp
- Targeted COI Gene in mtDNA
  - Cycling conditions
    1. 94C for 3 minutes
    2. 95C for 1 minutes
    3. 45C for 1 minutes
    4. 72C for 1.5 minutes
    5. Repeat 2-4 34 times
    6. 72C for 5 minutes
Results

- molecular results found using FASTA sequences processed by BLAST analysis software
- Diamondback moth was not found within guano
- ND reported 17.8 inches of rain
- *Aedes vexans*-linked to dog heart worm
- *Ochlerotatus dosrsalis*-linked to westnile virus
- *Melanotus similis*-harmful pests that damages potato, corn, and soybean crops.
Conclusion

• Amazing experience
• Confidence in my project
• Future research
Acknowledgements

• A sincere thank you to those who helped with my research this summer finding locations, trapping, extraction and PCR!!
• My mentor Mandy Guinn
• JP Holmes
• Dawn Alkafaji
• Marlee Finley
• Tahnee Herrera
• Tony Walking
• Josiah Nelson
• Rebekah Olson
• Mark Runyon
References

- Marsden, D. A. 1979, Insect Pest Series, No. 6 Cabbage Worm. Cooperative Extension Service, College of Tropical Agriculture and Human Resources.
- Olival, Kevin J.; Hayman, David T.S. 2014. "Filoviruses in Bats: Current Knowledge and Future Directions." Viruses 6, no. 4: 1759-1788