How DNA Methylation Inhibitors Affect Diapause in Megachile rotundata Keegan Foster¹, Josh Rinehart², Joe Rinehart³, Julia Bowsher² ¹University of Rhode Island, ²North Dakota State University, ³United States Department of Agriculture

Introduction

The alfalfa leaf cutting bee (*Megachile Rotundata*)(ALCB) is a solitary bee that when managed, proves to be vital for the alfalfa seed production industry of North America

-In the *Megachile Rotundata* life cycle, bees that overwinter are called diapausers. However, during the early season, there is an increased chance to having non-diapausing bees (bees that do not overwinter) which will result in a second generation.

-Farmers can run into issues with *Megachile Rotundata* management and alfalfa pollination due to the presence of these non-diapausers. With their early emergence, less bees are present in the following spring to pollinate alfalfa fields for seed production.

-By dosing Megachile Rotundata with the methylation inhibitor 5-aza-2deoxycytidine, we hope to see an overall reduction in the amount of nondiapausers present.

Hypothesis

5-aza-2-deoxycytidine will increase rates of diapause in longer photoperiods.

Methods

Dosage Determination:

- To determine the toxicity of 5-aza-2-deoxycytidine and in turn an appropriate dosage to be fed to our field study Megachile Rotundata

-Based on overall high survival rate and sufficient flight performance, the medium concentration (10 μ M) was chosen for the field study



feedings.

Non-diapauser



Figure 4. Pulled straw from a bee box containing 1 non-diapausing bee and 4 diapausing bees. Nondiapauser resembles an almost fully-fledged bee (outlined in green). Diapausers are still in their larval stages (outlined in purple).

P-value = 0.011 Z-score = 2.5390.25 0.00 Control Treatment

Figure 1. Male Alfalfa Leafcutting Bee.



Figure 3. One of six bee boxes used in the field portion of this experiment.

compared to the control bees occurrences

Future studies: maximize bee populations with a methylation inhibitor reduce their occurrence

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1) Pitts-Singer T. L., Cane J. H. 2011. The Alfalfa Leafcutting Bee, *Megachile rotundata*: The World's Most Intensively Managed Solitary Bee. Annual Review of Entomology. 56, 221-237. 2) Pegoraro M., Bafna A., Davies N. J., Shuker D. M., Tauber E. 2016. DNA methylation changes induced by long and short term photoperiods in *Nasonia*. Genome Research. 26, 203-210.



Results

Treated Bees Experience Higher Rates of Diapause			Treated Bees Exhibit Higher Rates of Pollen Ball Occurrenc		
P-value = 0.011		1.00 - P-value	= 0.005		
Z-score = 2.539		Z-score:	= 2.784		
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		O 0.50 -			
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Control	Control Treated Treated		Treatment		

Conclusion

- Bees treated with 5-aza-2-deoxycytidine experience a higher rate of diapause

- Compared to the control bees, treated bees exhibit a higher rate of pollen ball

- Our Findings correlate to results of previous studies and may suggest that this relationship can be found in a variety of insects

- Focus on finding a dosage to yield the highest diapause rates and lowest death rate to

- Administer widespread management practices that involve farmers dosing their bees

-Determine the importance of increased pollen balls with treated bees as well as how to

Acknowledgements

