# Buffet: In-Vitro Rearing of Alfalfa Leaf-Cutting Bees on Artificial Diets





**NDSU** 

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 $X^{2}$  (5, N = 1,672) = 55.6 p = <0.0001

# Alfalfa leaf-cutting bees rear young in enclosed brood cells

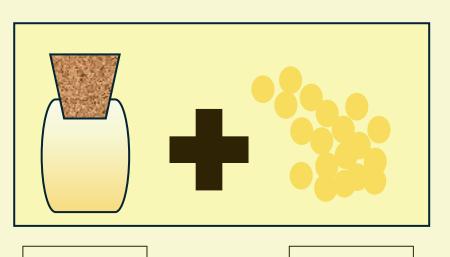
- Alfalfa leaf-cutting bees (Megachile rotundata) are a solitary species who rear their young in narrow cylindrical cavities
- Each brood cell contains a **provision** upon which the egg is laid consisting of nectar (protein/lipid) and pollen (carbohydrate)
- A consistent artificial recipe has yet to be produced for in-vitro rearing and is necessary for diet modification in laboratory trials and easier observation of developmental progression

Due to the similar makeup and viscosity, it is predicted that the Beltsville plus Prosweet diet will efficiently rear healthy bees

**First** 

Egg

**Brood Cell** 



Pollen

**x12** 

24-Well Plates

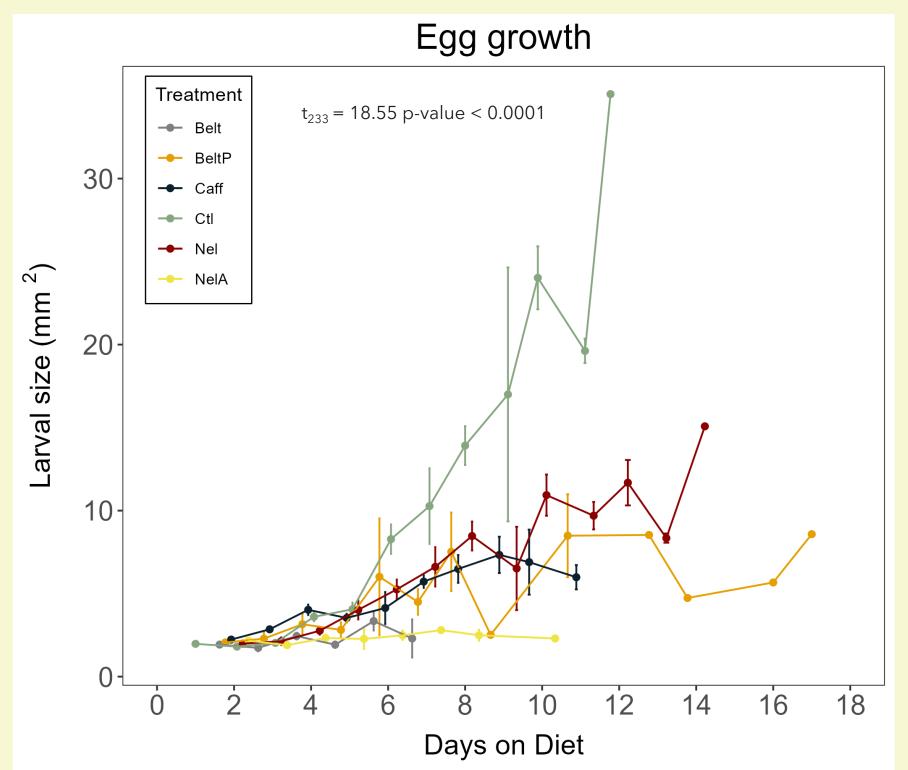
Days on Diet

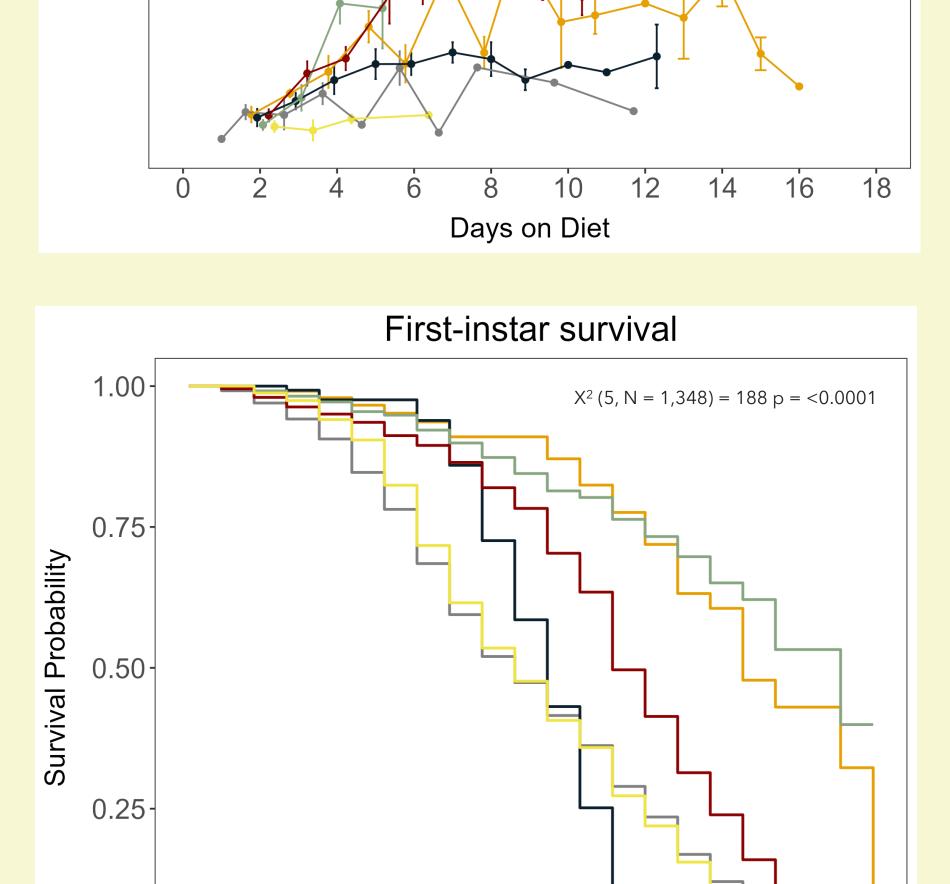
Egg survival

 $t_{233} = 18.55 \text{ p-value} < 0.0001$ Days on Diet

The control diet performed drastically better than diets comprised fully of artificial ingredients and those with naturally derived ingredients. The Nelson diet, containing pollen, accounts for the protein deficit found in other diets; whereas the Beltsville plus Prosweet diet provides the adequate sugars needed for survival but lacks the protein crucial for comprehensive web-spinning.

# Artificial diets perform poorly compared to those with naturally derived components





First-instar growth

 $t_{256} = 13.2 \text{ p-value} < 0.0001$ 

# Which diet consistently produces healthy bees?

#### Length & Width



## Transferred eggs/first instars from leaf pods into each diet

- In preparation for the experiment, 24well plate inserts were 3D printed to imitate natural conditions
- Eggs and first-instar larvae removed from broods, x-rayed, and placed onto 6 different diets
- Two 24-well plates assigned to each treatment; one for first-instars and one for eggs at 288 wells total
- Plates stored in an incubator at 27°C with 75% humidity
- Growth rate and survival observed 3x a week, recording length, width, and movement
- A linear mixed effects model was used to determine growth rates and a log-rank test was used to determine survival

### Further Questions

- Knowing pollen is the main carrier of protein, what would be an optimal protein substitute for pollen?
- Since the effect of caffeine was not further studied, how does caffeine affect lipid store consumption?
- If a diet is established, which macromolecular composition is ideal for rearing healthy bees?
- How would in-vitro reared bees perform on a drop-test or a morphological comparison with naturally reared bees?

#### Acknowledgments

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Thank you to Madison Floden, Marnie Larson, and Kendra Greenlee for technical support and guidance; Dr. Quinn, Dr. Baynham, and the USDA NextGen program for financial support; my fellow interns for moral support and camaraderie.

**Contact / Reference** Information

