**Digestion Assay Project Design**

We have *two treatments*:

1) **Diapause** (Long daylight hours, “winter”)

2) **Non-diapause** (Short daylight hours, “summer”)

Within each treatment, there are *two strains of ECB*:

1) **Univoltine (UZ)**

2) **Bivoltine (BE)**

There are **48 larvae per cohort**. **30 of these larvae will be sampled per cohort *AT THE START OF FIFTH INSTAR.***

How larvae will be sampled:

1) Remove old plant material, frass, and filter paper.

2) Place larvae into cup and weigh **Dry Mass** of larvae. **(Are larvae being weighed at start of fifth instar or when they are being sampled?)**

3) Place new filter paper into well and add a few drops of water for moisture.

4) Cut and **weigh new plant material** into well.

5) Track larvae based on their sampling group.

* 10 larvae will be ***sampled immediately upon entering the fifth instar.*** These will not go back into wells with fresh plant material.
* 10 larvae will be ***sampled at the midpoint of their fifth instar***. *(5 days for diapause, 3 days for non-diapause)*
* 10 larvae will be ***sampled at the end of the fifth instar.*** *(10 days for diapause, 6 days for non-diapause)*

6) When larvae reach their sampling date (date to be removed from plant material), place all the larvae for that sampling group (all 10 larvae) into a single microtube.

7) Place microtube with larvae into freeze dryer for ~3 days (exact time to be determined still)

8) **Weigh frass and remaining plant material** in well.

9) Remove dried larvae from freeze dryer and perform extraction.