

## Experimental Protocol: Diapause Incidence

The thermal conditions larvae experience in the incubators at UF and the USDA need to be confirmed and eventually compared between the two locations. To make that comparison we need to ensure the equipment we use to measure temperature are responding similarly.

1. HOBO comparison and calibration
  - a. Co-locate hobo's used to track temperature and humidity in incubators at UF designated for "Diapause" conditions
  - b. Co-locate hobo's used to track temperature and humidity in incubators at UF designated for "Non-Diapause" conditions
  - c. Co-locate hobo's used to track temperature and humidity in incubators at USDA designated for "Diapause" conditions
  - d. Co-locate hobo's used to track temperature and humidity in incubators at USDA designated for "Non-Diapause" conditions
  - e. Compare hobo temperature and humidity output between diapause and non-diapause incubators across 24-hours at both locations

If the abiotic conditions are similar between UF and the USDA, then a comparison of larval development time needs to be conducted to ensure there is no discrepancy between the development time of larvae at each location.

2. Track development time
  - a. 50 BE larvae will be reared using USDA and UF incubators.
  - b. The date the eggs were laid will be **recorded** and compared to;
    - i. Larval hatch date
    - ii. 5<sup>th</sup> instar date
    - iii. Wandering phase date

Those larvae that reach the end of the 5<sup>th</sup> instar under diapause and non-diapause conditions will be subjected to a 4 hour assay to determine the beginning of the wandering stage.

3. Assay for wandering stage.
  - a. Putative wanders (late 5<sup>th</sup> instar larvae) will be determined by tracking frass production across 4 hours
  - b. The number of days from the beginning of the 5<sup>th</sup> instar until the wandering stage will be **recorded**

Metabolic activity of wandering larvae that experienced non-diapause conditions and wandering larvae that experienced diapause conditions will be compared using respirometry.

4. Respirometry of late 5<sup>th</sup> instar larvae
  - a. CO<sub>2</sub> production of larvae in non-diapause conditions will be tracked every 2 days after the onset of the wandering stage and until they reach the adult stage.
  - b. CO<sub>2</sub> production of larvae in diapause conditions will be tracked every 2 days for the 40 days following the onset of the wandering stage.