**EAB Parasitoid Overwintering Project**

**Introduction**

* This experiment will be conducted in Lab 218 of the Marsh Life Science Building to study cold hardiness, supercooling points, and lower lethal temperature limits of *T. Planipennisi* and *S. Galinae*, both commonly used in biocontrol programs to reduce the population of the Emerald Ash Borer.

**Physical Standards**

* Main storage location for project will be rate-controlled lab incubators located in Alison Brody’s Lab and Brent Lockwood’s Lab next door to the Gotelli Lab (MLS 218). They are roughly 3 x 4 x 6 ft.
* Incubators will be kept at 25 degrees Celsius with a 12:8 L:D cycle as listed in the methods below.
* Incubators have been scheduled and reserved for George Ni, so no other individuals are expected to interfere with the machines, keeping temperature and humidity relatively stable (barring intentional disturbances from George or hired lab members)
* Additional signage will be posted to notify individuals against removal of biocontrol samples.
* Materials will be cleaned and sterilized per laboratory standards.

**Required Equipment**

* Storage space-kept at 4 degrees Celsius, located within the Gotelli Lab (MLS 211).
* 1.5 ml microcentrifuge tubes-enough to hold each individual sample (parasitoid specimen)
* Plastic Dowel-same number as microcentrifuge tubes
* O-ring
* 1.27-mm diameter (36 AWG) type-T copper constantan thermocouple for temperature readouts
* Ethylene glycol-goes down to -24 degrees Celsius
* Special Polycool liquid goes down to -30 degrees Celsius
* Kimax glass test tube
* Plastic Rack
* Rate controlled refrigerated incubators-Currently have 3 incubators (2 from Alison Brody’s lab, 1 from Brent Lockwood’s lab in MLS Building)
* Petroleum Jelly, Heat sink compound, or vacuum grease

**Methods**

1. Samples are first stored at 4 degrees Celsius
2. Chilled in batches of 20 larvae-each batch was at one of 3 cooling rates
3. 3 of 20 larvae in a batch are left at room temperature to serve as controls
4. The remaining 17 larvae in a batch are randomly assigned temperatures between 0 and -32 degrees Celsius, in 2 degree increments.
5. Insects are placed within 1.5 ml centrifuge tubes, a plastic dowel fit with an O-ring is placed in the opening.
6. A thermocouple is run through the dowel to make contact with the insect.
7. Microcentrifuge tubes are then placed into a 18 x 150 mm Kimax glass test tube and then placed in a plastic rack in silicon bath for cooling
8. Thermocouple readouts measure the appropriate chilling temperature
9. Cooling is done through a refrigerated circulator in a bath of silicon 180 oil (or ethylene glycol/special polycool depending on temperature requirement)
10. Once insects reach designated low temperature it is returned to storage at 4 degrees Celsius for three days.
11. During the three days they are checked daily for signs of discoloration.
12. After the three days, larvae are transferred to an incubator at 25 degrees Celsius with a 12:8 L:D cycle for 4 weeks.
13. These are checked and recorded for successful development to adult life stages
14. Supercooling points of samples are estimated using visual inspection of plots of temperature recordings from the lower temperature assay for evidence of an exotherm.
15. Response Variables- for LLT, discoloration of larvae after three days, or successful eclosion to adult stage
    1. For SCP, exotherm.

**Super Cooling Point**

* Defined as the lowest temperature recorded prior to the initiation of the exotherm
* With 20-30 individuals we can get a pretty good distribution of the Scps

**Lower Lethal Limits**

* Proportional lethal temperature
* Must account for differing cooling rates that exist in nature
* Approximately 5 temps spanning the range from 0-100% mortality to determine LLT50. More needed to get finer scale estimates

**Notes, Tips, Pre-Start Equipment**