**Warming Climate Extremes SBB Growth Experiment\***

*\* only temperature*

**Background & Notes:**

* By 2080 (+60 yrs from now), Miami’s climate, if high emissions are not reduced, will be **6.3 C warmer** and **91.5% drier** (University of Maryland Center for Environmental Science = [UMCES](https://fitzlab.shinyapps.io/cityapp/), [Fitzpatrick & Dunn, 2019](https://www.nature.com/articles/s41467-019-08540-3)). If high emissions are reduced, then Miami’s climate will be **3.4 C** warmer and **42%** drier by 2080.
* Possible temperature regimes:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Regime Symbol** | **Realistic (*a priori*)** | **Less realistic but evenly spaced** | **Least realistic but retains extreme trial format** | **RH** | **location** |
| **A (control)** | 28 C | 28 C | 28 C | 70% | Potting room |
| **B (stagnant)** | 31.4 C (high emission reduction) | 32 C | 36 C | 70% | Atrium 1 |
| **C (stagnant)** | 34.3 C (continued high emissions) | 36 C | 40 C | 70% | Atrium 2 |
| **D (temperature variability)** | Daily heat waves (28 C low and 34.3 C high) for 3 days / week. 28 C for 4 days/week. | Daily heat waves (28 C low and 36 high) for 5 days/week. 28 C for 2 days/week. | Daily heat waves (28 C low and 40 C high) for 5 days/week. 28 C for 2 days/week. | 70% | Manually move the bugs from atrium incubator to potting room incubator at certain times of the day or for certain days. |

* Mean eggs laid between a two week span for 2021 Summer field collected bugs was 27 eggs.
* Extreme trial long-wing numbers around 230-250 individuals.
* LW = “long-winged”
* SW = “short-winged”

**Main question(s)**

* How do **extreme hot** temperatures and extreme temperature **variability** affect SBB phenology, development, and/or morphology?

**Protocol**

1. Mate flight-tested SBB\*. Picked **KL population** because there are at least 5 mothers to pick from and 2 of them have enough offspring alive to generate 25 or more mating pairs. PK had 4 mothers but only 1 of them have 25 or more offspring. Randomly select two mothers with more than 25 offspring (i.e. 114 and 16). Within those two mothers, randomly select **25 LW males** and **25 LW females\*\*** (*males from one mother that laid more than 25 viable males and females from another mother that laid more than 25 viable females*) no more than about **1 week apart in age.** Randomly assign nonsibling pairs (**pairs\_final.csv**). (Q: How long does it take for a fem SBB to start laying fertilized eggs after mating? How to mate SBB for fertilized eggs (e.g. for how long? How to monitor?)? How many pairs and mating tries are needed to get upwards of 650 eggs (viable or inviable)?)

\*Ideally, I was looking to filter for only those tested at 28 C and 70% RH, but there would not be enough numbers. If minimizing genetic variability is not important, then another method would be pairing up males and females from the same population for those only tested at 28 C. However, there are only 37 bugs in that pool (24 are KL and 13 are PK).

\*\*There are 23 F and 26 M from 114 and 16 F and 36 M from 16. So I dropped the number down to 20 pairs. Pairs are generated in **pairs.R script** and its output is **pairs\_final.csv**.

1. Set up 20 large bug homes for mating on Nov 1. Mates will be incubated at 28 C and 70% RH, and have 2 GRT seeds: 2 BV seeds: 2 water picks refreshed each week.
2. Collect eggs between Nov 1 and Nov 15 2021 from first-generation SBB pairs. I estimated that between two weeks each pair will lay 30 eggs based on previous data. Eggs will go in tubes labeled by ID starting in the 2000s (e.g. 2000, 2001, 2002, 2003…).
   1. Record in **gen2 masterlist** datasheet up to **650 entries** with the following new columns: **temp, RH, and instar date & masses** (see datasheet folder).
3. Randomly assign a temperature regime ( A,B,C,D using =INDEX($<col>:$<col>, RANDBETWEEN(1, COUNTA($<col>:<col>)),1) in Excel sheet **gen2\_masterlist.xlsx**) to each egg.
   1. A, B, C, D are stand-ins for potential temperature regimes. See above.
4. Once eggs hatch, grow nymphs in individual bug homes (1 GRT: 1 BV seed, water pick). Record the **instar** **emergence** **date** and **mass** for each instar stage. It takes generally 1-1.25 months for the nymphs to become adults, which will lead up to the winter holidays.
5. Upon the first hatch, care would occur in two weeks. Then, care would regularly happen weekly.
6. Take morphology measurements of adults.
7. \*\*\*

**Timeline**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Dec 27-29** | **Nov 1- 15** | **Nov 16 – Dec 23** | \*\*\* |
| **Morning** | \* Extreme trials | \* Extreme trials | \* Monitoring instar growth & massing  \* Care |  |
| **Afternoon** | \* Experiment Prep | \* Egg collection | \* Flight Paper 1 |  |
|  |  |  |  |  |

**Materials** (Q: Will lab funds support this project?)

* 3 [temperature data loggers](https://www.amazon.com/Elitech-RC-5-Temperature-Reusable-Recorder/dp/B074PNM4RV/ref=zg_bs_5006549011_2?_encoding=UTF8&refRID=82NGD5EG9YJ1TY78WYQT&th=1) ($78 each + shipping)
* A fine-tuned mass scale for nymph massing
* 20+ big bug homes
* ~450 bug homes prepped
* Flip cap tubes
* GRT seeds (~2,700)
* BV seeds (~2,700)
* 3 [cameras](https://www.target.com/p/polaroid-sport-action-camera-720p-red/-/A-79785378?ref=tgt_adv_XS000000&AFID=google_pla_df&fndsrc=tgtao&DFA=71700000023621483&CPNG=PLA_Electronics%2BShopping_Brand_Competitor%7CElectronics_Ecomm_Hardlines&adgroup=SC_Electronics&LID=700000001170770pgs&LNM=PRODUCT_GROUP&network=g&device=c&location=9021740&targetid=pla-323438499111&ds_rl=1246978&gclid=CjwKCAjwzt6LBhBeEiwAbPGOgcd2AXTyPcxOVw8aKVJyfu-XvpX74lMlqYqaEdFa8dQx43ctnQqfwBoCmx8QAvD_BwE&gclsrc=aw.ds) & a [micro SD card 512 gb](https://www.amazon.com/Memory-Transfer-Adapter-Surveillance-Security/dp/B09CM58TRC/ref=sr_1_1_sspa?dchild=1&keywords=512gb%2Bmicro%2Bsd%2Bcard&qid=1635295282&sr=8-1-spons&spLa=ZW5jcnlwdGVkUXVhbGlmaWVyPUExRDJSMlRFUEQ5ODFZJmVuY3J5cHRlZElkPUEwMzA4ODY3VTVaMloxNVdSWUNXJmVuY3J5cHRlZEFkSWQ9QTA4MDkyNDUxVDVGTTRTU01YVEZWJndpZGdldE5hbWU9c3BfYXRmJmFjdGlvbj1jbGlja1JlZGlyZWN0JmRvTm90TG9nQ2xpY2s9dHJ1ZQ&th=1) & [card reader for Mac](https://www.amazon.com/SmartQ-C368-Multi-Card-Compatible-Supports/dp/B06Y1G18KS/ref=sr_1_1_sspa?dchild=1&keywords=card+readers+for+mac&qid=1635295425&sr=8-1-spons&psc=1&spLa=ZW5jcnlwdGVkUXVhbGlmaWVyPUFBUlQ1M1pBOTVXT0omZW5jcnlwdGVkSWQ9QTAyMzE1MTBaQ0UxRDdMUkdXVEomZW5jcnlwdGVkQWRJZD1BMDc4Njk1NkNSNE9KSzVQWFNNUCZ3aWRnZXROYW1lPXNwX2F0ZiZhY3Rpb249Y2xpY2tSZWRpcmVjdCZkb05vdExvZ0NsaWNrPXRydWU=) ($164 + shipping)
  + For comparison, Apple iPhone 5 has 8 megapixels and it’s still able to capture insects on it.
  + Can magnetically mount these cameras on a location to monitor egg hatching and nymph growth over the weekends. This can be a good foundation to collecting and processing video data.
* Total cost (excluding seeds) = ($242 + shipping for 4 items)

**Measurements**

* Eggs laid (viable vs. inviable) by the mated adults
* Larval mortality/survival
* Mass
* Instar dates (e.g. emergence day, coefficient of variation in emergence for each temp regime)
* Flight morphology
* Record video over the weekends (quantify stress based on insect movement in a set of X randomly selected bug homes (X = number of max cups that can fit in the screen))
* \*\*\*(After holidays so not possible given the timeline) collect inviable eggs for 2-4 weeks as adults to measure fecundity and/or flight test all (same age) at 28 C.