**Flight Trial General Protocol**

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There are two visible flight behaviors that soapberry bugs (SBB) exhibit: bursting flight and continuous flight. From previous flight trials, we have defined each category as the following:

***bursting*** - bug flew but did not maintain flight behavior for at least 10 minutes

***continuous*** – bug maintained flight for longer than 10 minutes

During a flight trial, bugs could exhibit both bursting and continuous flight. These definitions factor into how flight trials are conducted.

**Procedure:**

**Incubator conditions:** 28 C, 70% RH, 14:10 (L:D) 8 am sunrise and 10 pm sunset

**day before**

1. Pull 50 bugs if using 1 flight mill and 100 bugs if using 2 flight mills, following the datasheet. The datasheet has randomized ID bug testing order.
2. Then, use magnetic paint to paint each bug’s pronotum. Bugs are attached to the flight mill magnetically.
3. Add sugar water in a 2.0 mL microcentrifuge tube made from 7 volumes of Fruit Punch Gatorade and 3 volumes of DI water and stoppered with cotton.

**trial day**

1. Turn on the flight mill incubator (ON/OFF switch on the upper side of the incubator), the laptop, and the solderless breadboard switches.
2. Gather bugs for testing (could be stored in a separate incubator). SBB are located in individual bug homes within labeled large boxes. Recommendation: sort bugs by color, then order them by ID according to the datasheet.
3. Place the first 8-16 ordered bugs on labeled plastic box lids. Plastic box lids are labeled to match the chamber letter and number of the flight mill. For example,

|  |  |
| --- | --- |
| Flight mill cells | |
| A-4 | B-4 |
| A-3 | B-3 |
| A-2 | B-2 |
| A-1 | B-1 |

|  |  |
| --- | --- |
| Plastic box lid | |
| A-4 | B-4 |
| A-3 | B-3 |
| A-2 | B-2 |
| A-1 | B-1 |

1. For the remaining ordered bugs, place them on non-labeled plastic box lids for easier transport between incubators or for massing.
2. Mass the bugs on a scale before beginning flight trials. Use an empty saucer cup and tear the scale to take the mass. Record each mass on the datasheet. Recommendation: if operating with 1 flight mill, weigh 30 bugs at a time. If operating with 2 flight mills, weigh 60 bugs at a time.
3. When bugs are ready to be flight tested, set up a new recording session for both A and B chambers/data loggers. Follow “To start a **new** Recording Session” description under the “**Using WinDAQ on the Dell Computer”** protocol.
4. Start loading the bugs on the flight mill and record the bug’s set number, chamber letter and number, and test time and date on the datasheet. Once the first bug starts flying 1.) Press “OK” to start recording and 2.) record on the data sheet if they flew “Y” for yes or “N” no.
5. Once all the bugs have been set up and each has been motivated to fly, start a timer. In 10 minutes check on the bugs and motivate them to fly again. In the next 10 minutes, motivate the bugs to fly again. In total, this will give the bugs 3 attempts to fly. Then, at the 30 minutes mark, check on the bugs. Those bugs who had not been flying continuously (only bursting) since you’ve motivated them 3 times, can be taken down. Those bugs who are flying continuously since their last attempt can say and be given a ‘cap’ marker to signal that they should not be removed.
6. Replace the bursting bugs and any continuous, long flying bugs who suddenly stopped at each 30 minute mark. Replacements occur at every 30 minute interval with a new set of massed bugs. Before taking bugs off, ensure to write down the time and date you replaced them and, for continuous bugs, write down the time you saw the continuous bugs stop flying.
7. When new bugs are ready to be loaded following the first set of bugs, create an event marker with the bug’s ID in the marker’s description. Follow the “To make an **Event Marker/Comment** during a Recording Session” description under the “**Using WinDAQ on the Dell Computer”** protocol.
8. Continue massing and loading bugs until all prepped bugs for the day have been tested.
9. Turn everything off that was initially turned on in step 4 when done, **unless** bugs are still flying continuously. If they are and the file is running out of space at the end either A.) create a new recording session and keep the bugs flying overnight or B.) append to the existing file. Follow “To **Append** to an already existing Recording” description under the “**Using WinDAQ on the Dell Computer”** protocol.
10. Place tested bugs back into their plastic box within the incubator and repeat steps 1-3 for the next set of bugs.