**Bursters Protocol**

First Created: Nov 7, 2019

Updated: Jan 31, 2020

**Why are we doing this trial?** Now that we have tested all the soapberry bugs that definitely show flight behavior, we filtered them further into those who only burst and did NOT show continuous flight this week. So we want to double check if any of these bursts will fly continuously. Basically, we want to give them another chance to fly continuously because who knows if they will or if a burster is always a burster.

**Our definitions:**

**‘Bursters’** are soapberry bugs that can fly as briefly as 1 second to less than 10 minutes. Any noticeably flying behavior that lasts less than 10 minutes is considered a burster, especially after given 3 attempts.

**‘Continuous’ or ‘long’ flyers** are soapberry bug that will fly for 10 minutes or greater continuously after their *last* attempt.

**What does this look like in action?**

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**Procedure:**

1. First, turn on the flight mill incubator (switch on the side of the incubator, high up!) and turn on the laptop, solderless breadboard switches
2. Then, gather the bursters from the main incubator. They are located in a large box labeled ‘Bursters of the day”. I recommend sorting them by color to save time down the road. Then order all of them by number, organizing them on the blank (non-labeled) big plastic box lids.
3. Separate the first 16 bugs on their box lids and place the rest of the bugs on their box lids and place them in the incubator for easy access and to keep them warm!
4. Take those first 16 bugs on their box lids and take their mass on the scale in the lab next door. Use a saucer cup and tear the scale to take the mass. Record each mass on the datasheet.
5. Then, take the 16 bugs, place them next to the laptop in the flight mill incubator and set up your **new Recording Sessions** for both the A and B chambers/data loggers. Follow “To start a **new** Recording Session” description under the “**Using WinDAQ on the Dell Computer”** protocol.
6. Start setting up the bugs on the flight mill and record at what time and date each bug was placed in their chamber. 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.
7. 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 tries to fly. Then, at the 30 minutes mark, check on the bugs. Those bugs who had not been flying continuously (aka. just bursting) since you’ve motivated them 3 times, can be taken down. Those bugs who are flying continuously since the last attempt can say and be given a ‘cap’ marker to show that they should not be removed.
8. Replace the bursting bugs and any continuous, long flying bugs who suddenly stopped at each 30 minute mark. Write down the time you saw the continuous ones stop flying and replace them. **\*Before you replace bugs**, write down the time and date you replaced them and create a **commented Event Marker**. Follow “To make an **Event Marker/Comment** during a Recording Session” description under the “**Using WinDAQ on the Dell Computer”** protocol.
9. Repeat steps 6-8 (\*\*but no need to start a new recording! It will already be running).
10. Turn everything off 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.