

Extreme Trials Protocol

Nov 4, 2021

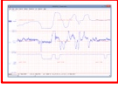
Adjusting the Temperature on the Walk-in-Incubator

1. Edit the incubator program titled “**Soapberr**” so that its conditions match the desired experimental design. The time, lighting, RH, and temperature of the program can all be adjusted. Generally, incubators are set at 10 hours light and 14 hours dark with the lights turning on at 8 am and the lights shutting off at 10 pm.
 - a. To change the temperature and/or RH, press **Program**, find the **Soapberr** program by pressing the up and down **arrows**, and hit **Enter** twice on the desired program.
 - b. Continue to scroll through the program’s steps to orientate yourself. The user will see two steps, one set at 8 am and the other set at 10 pm.
 - c. Edit the 8 am step first, making sure the RH is 70% and the temperature is the desired temperature regime for the day.
 - d. To edit a step, hit **Program** and scroll down with the **arrows** to the **Edit Step** feature and hit **Enter**. On the new screen, scroll down to the temperature, hit **Enter** to begin editing. Use the **arrows** to adjust the temperature higher or lower.

Note: A temperature change of 28 C to 40 C can take as long as 30-45 minutes to properly adjust.
 - e. When finished, scroll all the way down to the end of the editing step and it will pop you out back into the screen where you can **add, edit, or delete a step**. Scroll down again using the **arrows** and hit **Quit**. This will pop you back into the main screen.
 - f. To run the new adjustments, hit **Program > Run Program >** and select **Soapberr**.

Turning on the Dell and Testing the WinDAQ Software

1. Press the power button in the top right hand corner of the keyboard.
2. When the screen powers on and loads, there are three situations you can face yourself in.
 - a. The first is the login screen, where you input the **labadmin password, dispersal202**, which is written on the post-it note on the laptop keyboard.
 - b. The second is a blue screen that will ask for a **bitlocker recovery key**. That is also written at the bottom of the post-it note. Once you enter the recovery key, then you will be able to enter the **labadmin password**.
 - c. Finally, the laptop could state the **old password expired** and you need to update it. You can simply recycle the same password, **dispersal202**, when it asks for that.

- Once you're in the laptop, launch the WinDAQ Software, which looks like a blue waveform with a red grid ().
- On the new popup window that loads, the four dataloggers should be read and **available**. They are available to be launched if you're able to input checkmarks in the boxes. If you can't, then they are "**busy**" and you may need to disconnect the datalogger from the laptop and reconnect and/or restart the laptop.
- Four new popup windows will appear, one for each datalogger. The **letters on the flight mill chambers correspond to a data logger and its serial number** which are labeled on the physical rim of the laptop. Split the laptop screen between each running logger and next to its labeled serial number. Notice also that each chamber specifically corresponds to a data logger channel, but the order is inverted. For example, A-1 is on the bottom row of the flight mill, but it is the top channel for the data logger. Keep this in mind.
- Test that each channel is running smoothly. Hover the flight mill arms and give each a spin while checking that **voltage dips are being recorded** at each revolution. If voltage dips are not being recorded, skip the channel and/or ask for assistance.

Missing Bugs

- Order the bugs** according to the ID order on the datasheet. Place the ordered bugs on the plastic boards labeled with either the chamber letters and/or numbers.
- Press down on the handle on the analytical balance to turn the balance on. Place the mass cup on lid and **zero the balance** by also pressing down on the handle again.
- Place each bug** in the mass cup and record each bug's **mass**. Also, note whether a bug has **eggs laid** in its cup on the data sheet.

Flight Testing

- On the datasheet, record which bugs are going in which chambers. Also, record the set number.
- Begin a recording file on each data logger. Click on the window of that data logger you want to start recording in, then **File > Record. Go to Documents >**. Scroll down to the latest recorded file and click on it, this will autofill the filename of the new recording with the latest filename. Update **the filename with the new set number, date, and, where necessary, update the chamber letter**. Hit **OK** and then update the time field to be **24:00:00** or 24 hours. Hit **OK** and repeat this step for each data logger.
- Begin loading the bugs, one by one, in their corresponding chambers:
 - For each bug, record whether you saw the bug fly or not (Y or N), then define its flight characteristics. There are two flight types:
 - Bursting: bug flaps wings intensely for 2 sec and flies within the 30 minute interval but not beyond the 30 minute interval. Record a "**B**" in the flight type column on the datasheet if you see this behavior.

- ii. Continuous: bug flies beyond the 30 minute interval. Record a “C” in the flight type column on the datasheet if you see this behavior.
- b. For each loaded bug, give it a burst of “**wind**” from the hand-held fan. This is the bug’s first flight attempt. **Each get 3 flight attempts total.** It will go like this:
 - i. Wind count: 1
 - ii. Time: 0 min
 - iii. 10 minutes pass
 - iv. Wing count: 2
 - v. Time: 10 min
 - vi. Another 10 minutes pass
 - vii. Wind count: 3 = LAST TIME
 - viii. Time: 20 min
 - ix. Another 10 minutes pass. Remove any bugs that have stopped flying or are not flying continuously. Keep the bugs that are flying continuously on the flight mill and make place a small piece of post-it note on the chamber cell to remind yourself.
- 4. If there are more than 16 bugs being tested, continue loading bugs then ask for assistance. But for now focus on the less than 16 bugs being tested.

Saving the Recording File and Shutting Down Equipment after Trials are Over

1. **Click on the window** where there is no more flight movement happening.
2. On the keyboard **hit Ctrl + s, to save the file.** Do **not** do this motion when bugs are still flying. If you make the mistake of doing that, then you can quickly append a new file. To do so, follow the motions of recording a new file and then click on the filename that you accidently stopped running. Press OK and when it tells you that the file exists but also asks if you would like to append to the file, click OK.
3. Once all the bugs have stopped lying and each file has been saved. **Turn off the dell and switch off the power strip** right in front of the laptop.

Finally, reprogram the temperature on really hot or cold days to a less energy intensive temperature like 28 C.