FAQ style methods and step-by-step instructions for bird-arthropod manipulations

Setup questions

**And are the controls (non-bagged plants) supposed to be a paired design, so they should be as close to their bagged counterpart as possible?**

Think of each bird-bag + nearby control as a pair that is treated as a single replicate in the experiment.

**Can we drape bird bags over plants instead of using the sock-shape bags?**

Only for host plant species in which the sock-shape bagging will not work (thorny shrubs). In this case, use the same size bagging (~7x7 feet) and fix the bag to the ground with tent stakes or to the branch base.

**How far apart should plants and bird exclusions be from each other?**

25m minimum distance of pairs from each other *of the same species*. If you have a nice set of cherry, barberry, and oak, for example, they can be near each other (we did this a lot in the bird-ant studies). Just make sure the next cherry is 25 meters away, for example.

Do not have bird bags closer than 5m to each. Having control and treatment close (5m) is good for the analysis.

**Can we set up some bags early?**

No, the two-week timing is important to follow based on the previous work. Longer duration for some bags means different treatment effect size. Those bags you set up will be really off the rest of the schedule and probably can’t be used in the full analysis.

**Do we need to sample trees in the same order they were set up?**

They should be sampled in roughly the same order based on the 6 time blocks. (A through F). So if you set up 20 plants on May 5, time block A, sample those same 20 plants first on the week of May 17 as time block A in its entirety. There is no need to sample the 20 plants *for that one day* in the same order they were set up; we never were that strict.

**How can we find the trees to resample after doing the set up?**

Please tag the trees with phone GPS during set up. To help find them again if they are off trail, also make a hand drawn map to show where trees are relative to each other and the trail. It’s not a true map or anything, just a way to jog the memory when you go back to resample. For example, it’s good to know that those random oaks you need to find were a thousand feet from the trail past the rest of the plants and there is a note of that.

Sampling period questions

**What is the basic insect sampling protocol once we first go back to the branches?**

At the start of sampling you need 2 beat sheets, 1 dowel, vials, aspirator, datasheet. A datasheet should be made ahead of time with the Tree ID and basic info about insect #s collected to keep track of what we got. Since ID’s will happen back in the lab no need to go into detail. The final dataset should look something like the attached document. We can make a much cleaner one in the future.

To sample the branch, position both beat sheets underneath before handling the branch and have one person with aspirators and vials in hand ready to go. Then grab the branch and move it over the sheet. Small insects like spiders and ants and possibly larger caterpillar may fall even before the “beat”. Be sure to collect them.

Now hit the branch vigorously 5 times, strong enough to shake the branch strongly but not enough to damage the bark of the twig. Use the aspirator to grab any small insects – nearly all ants and spiders for example fall on the first beat. Afterwards, hit the branch a second time to dislodge any recalcitrant caterpillars or hemipterans holding onto the branch.

Many flying insects are going to get away – flies, small wasps, moths, etc. Don’t worry. This just the flaw of the sampling method – it only collected foliage-feeding and sap-feeding herbivores and foliage-gleaning predators. It’s meant as a way to sample insects associated with the plant and also those that are likely to be targeted by searching predators like birds (ants, spiders, caterpillars, hemipterans, some beetles).

Collecting insects takes two strategies: hand collecting directly into vials and aspirating. Caterpillars, some ants, large spiders, etc. Can be “scooped up” into plastic vials directly from the sheet. Smaller, faster insects (small ants, spiders, beetles, hemipterans) need to be aspirated since they are too quick and numerous.

**What weather conditions can we sample in?**

Sampling can occur during daylight hours and on any temperature day. However, in heavy wind or rain conditions sampling is not advised. Insects are still present, but the aspirator does not work well when insects are wet. Light rain or cold days are fine, and actually the easiest for collecting because the cooler weather slows down the insects.

**What order should we sample in?**

Sampling order is extremely important, but only for a given day. So, for example, if you set up 20 branches on one day, sample those same 20 branches when you revisit. Always sample the paired control branch on the same day as the treatment and in the same order they were set up. Insect development times are extremely brief and weather dependent.

**How many vials or containers do we need?**

In a given day, you may need 10 or 20 vials for a single branch. Each vail will hold an insect or two. The problem is if you open them again to collect more, the originals you collected will come out. Therefore, at the end of the field day, you should label and freeze everything, then condense everything from a given branch to one bag or vial for long-term keeping. (Recycle vials from the field, in other words).

**How many branches can we sample in a day?**

You should not sample more than you set up a in the sampling period. If you have extra time, do not try to “catch up” and collect some stuff early. In past years, we would try to complete about 24 to 48 branches in a field day, but that is because we erected 24 pairs of bird bags and controls in the set-up period.

**How should we store insects in the field?**

Put insects in a cooler with a small ice pack to prevent them from “roasting” on hot, sunny days. If they are trapped in vials they can die and break apart very quickly. Make sure all vials are labeled before being put into the cooler.

Lab / specimen processing questions

**What data are most important from the arthropod community?**

Abundance of each species group. When in doubt, take a picture of the insect(s) with a tag in the background to show the specific host plant the specimen came from. We can always go back and figure things out from there.

**Ok, what do we do at the end of the field day?**

Everything from a given field day should go straight into a freezer overnight to freeze-kill and preserve insects. However, it is critical that every single bag/vial/jar is labeled clearly and with waterproof labeling. Never put an unlabeled insect sample in the freezer, it is a waste of time and will hamper processing steps.

**What do we do after the freeze step?**

This is when to condense the samples into a smaller number of containers. Vials and attachments for the aspirators are limited, and so is freezer space.

If time permits the next morning, one should remove samples from the freezer, and then sort them into major functional groups and place into small jars. Major groups include caterpillars, ants, spiders, beetles, and Hemiptera (true bugs, aphids, etc.). Make a note of the counts of these major groups in the raw field data.

**Where is the raw field data?**

There should be always two versions and they should be updated daily. A binder in the lab and then an electronic one updated at the same day. This should be stored as a shared excel .xlsx sheet on the Great Hollow Drop Box.

**How is the binder laid out?**

Instead of a lab notebook, we should use a 2-4 inch 3-ring binder with sheet covers and printouts of the “field data sheet”. These field data sheets are formatted for this specific experiment. Make any side notes or issues on this. The binder is for all the messy information regarding the field survey.

**How is the excel data laid out?**

“Branch data 2020.xslx” This data needs to be as clean and organized as possible. I have provided a template in the drop box. **There should be no blanks unless the data has not been entered.** **A true zero (i.e. no caterpillars on a given branch that was actually sampled) should always be entered as a zero.** A blank means that we did not sample the host plant, or we are excluding it from the analysis for some reason (a rare occurrence).

**How long can insects be out of the freezer of ID purposes?**

Never longer than 5 or 10 minutes. 5 minutes preferable. Just have a few out at a time and put them right back in the bag they came from. Thawing insects will degrade quickly making ID impossible and lost data on fatty acids or protein.

**Is this a spider or an ant?**

Don’t worry about that right now. Take a picture with the digital microscope attachment. Label and store the jpeg on the computer in the lab in a folder for that specific day.