Last updated Oct 12, 2023

Leaf Collection and Fall Gall Dissection Protocol for Sites

Please review this protocol carefully before proceeding with leaf collection.

Materials:

Provided by us:

- This protocol
- 50 tea bags
- 1-gallon ziplock bag containing 500g of silica desiccant beads (or multiple bags if collecting from multiple sites)
- 1 return label
- 10 2 mL microcentrifuge tubes with 95%+ ethanol (for collecting dissected insects)

You need to supply:

- Sharpie/permanent marker
- Clippers or scissors for fieldwork
- Packing tape for mailing the samples back

Site selection and plant identification:

1. Select sites with a sufficiently high density of *Eurosta* galls to collect, measure, and dissect at least 50 (ideally 200) galls during this spring. Your chances of collecting an adequate number of galls are higher when the host plant distribution forms a large, continuous stand.

2. Ensure that the selected sites have a low risk of mowing so that you can visit them during both this fall and spring (ideally, again in spring 2025, although this is flexible).

3. Determine the species of goldenrod at your site, using this key:
https://gobotany.nativeplanttrust.org/genus/solidago/

Leaf collection:

1. Sample a minimum of 20 stems (ideally 30) from each species. Ensure that the selected stems have *Eurosta* galls growing on them to confirm they are *Eurosta*'s host plants.

2. Space the sampled stems at least 2 meters apart to reduce the risk of repeated sampling from clones.

3. From each stem, collect five to ten fresh leaves, ideally without herbivore damage (see the diagram at the bottom of the protocol for differentiating between gall types). Place the leaves from each stem in a separate tea bag.

4. Put the tea bags into a gallon ziplock bag with silica gel. If your site has multiple host species, please ensure that leaf samples from different host species are placed in separate ziplock bags. Seal the ziplock bag securely.

5. Label the ziplock bag with the host plant species, Site ID, and the date.

Example:

Host plant: *S. altissima*
Site ID: NHJ
Date: 18/09/2023

Host plant: *S. altissima*	Label the bag with the species ID
Site ID: NHJ	Three letter uppercase site code you make. Before sending your samples back to us, you will fill out a form that will ask for your site(s) coordinates and three letter code(s)
Date: 18/09/2023	Day/Month/Year formatt DD/MM/YYYY

You do not need to mark plants that you sampled from, as we aren't tracking individual plants but just surveying your site.

Fall gall measurement and dissection (optional):

The measurement and dissection of a few fall galls serve two key purposes:

1. Preliminary Data for NSF Grant Proposal: These measurements and dissections will provide preliminary data essential for the NSF grant proposal planned for this fall.

2. Genetic Information Preservation: Additionally, this process acts as a precautionary measure to safeguard genetic information. In the event that the site undergoes changes next spring, such as mowing or other factors leading to its alteration or disappearance, this preserved data ensures the conservation of valuable genetic information.

Gall collection and measurement protocol:

1. Collect around 30 galls.
2. Measure the diameter of each gall at its widest point using calipers
3. Proceed to dissect the individual galls using garden pruning shears. Remove the insect, whether it is a larva or pupa (as we are interested in both stages), from within the gall.
4. Place the insect in a microcentrifuge tube of 1.5 or 2 mL capacity, which should be filled with 95%-100% ethanol. Ensure that gall fly larvae and any other natural enemies are placed in separate tubes and labeled accordingly. It is not necessary to match individual larvae with specific galls for our purposes.
5. For more comprehensive instructions regarding gall dissection and species identification, please refer to the gall dissection protocol (link provided).

If you've discovered multiple host plants with Eurosta galls on the same site or sites with non-*Solidago altissima* plants hosting galls, we want to emphasize that we remain highly interested in your findings!

These sympatric sites and the presence of *Eurosta* individuals associated with non-*Solidago altissima* host plants offer valuable insights for various aspects of research. For instance:

1. **Comparing Host-Associated Genetic Differentiation:** They can be instrumental in comparing host-associated genetic differentiation across the speciation spectrum, such as distinguishing between host races and separate species.

2. **Parallelism of Genetic Differentiation:** They enable the study of genetic differentiation patterns across different geographical regions, for example, the comparison of genetic differentiation between host race *Eurosta solidaginis* on *S. altissima* and *E. solidaginis* on *S. gigantea* in the Midwest versus New England.

3. **Outgroup Analysis:** More distantly related *Eurosta* species found in these sites can serve as valuable outgroups in genomic analyses.

4. **Co-Speciation Patterns:** The genetic information from parasitoids, if obtainable, can be used to test for co-speciation patterns between *Eurosta* and its associated parasitoids.

Therefore, your site remains exceptionally valuable for advancing our research in various dimensions.

The number of galls from non-*Solidago altimssima* golden rods you should collect:

If you have the time and the site contains a minimum of 10 galls associated with non-*Solidago altissima* goldenrods, we would greatly appreciate it if you could also dissect these galls and send us the insects.

Even obtaining genetic information from a few individuals sourced from these non-*S. altissima* goldenrod host plants will be valuable, serving as essential outgroups in our genomic analysis.

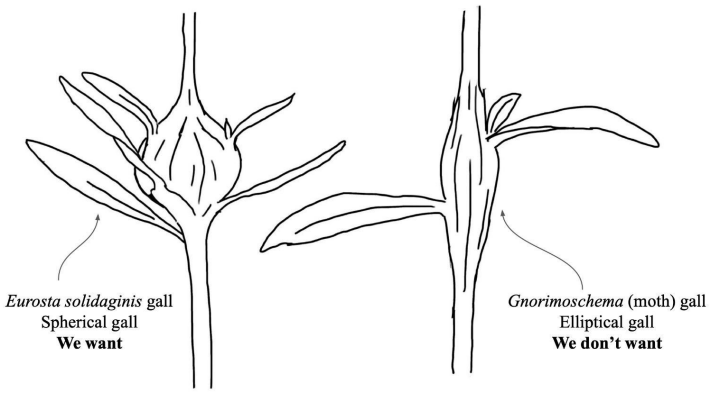
If it's possible to collect more individual insects (ideally 20 or more), that would be even more beneficial, as it would provide us with a substantial sample size to obtain robust estimates of population genomic parameters.The exact number of galls you need to collect to reach 20+ individual fly larvae depends on the local site and factors such as early mortality rates and parasitism rates. If feasible, we recommend collecting at least 100 galls to ensure you can obtain the desired 20 gall fly larvae.

The dissection protocol for these non-*Solidago altissima* galls is the same as the protocol for dissecting *S. altissima* gall (see above).

Sending samples back:

1. Place the microcentrifuge tubes into a quart sized bag (we provided) and label with site info and host species info as for leaf sample bag. Please do not place the quart sized bag inside the gallon zip lock bag in case a leak of ethanol occurs.
2. Place this bag into the shipping envelope.
3. Fill out and submit sample submission form https://forms.gle/3nUdLsZXQrFpKVRX9 (also linked in your kit confirmation email).
4. Tape prepaid return label over label on envelope used to send kit and drop off at FedEx or UPS
5. Expect an email letting you know when we've received your samples
6. For gall size data, please send it over email o.bronzomunich@gwu.edu.

We really appreciate your interest and involvement in this project! Again, please email o.bronzomunich@gwu.edu with any questions.



For this project, we are interested in the spherical galls formed by *Eurosta solidaginis,* known as ball galls. We are not interested in the galls formed by the goldenrod moth, which forms elliptical galls (football shaped). Make sure to select a site where the ball galls are common, and to dissect and measure ball galls and not the elliptical galls.