Last updated Oct 3, 2023

Leaf collection and fall gall dissection protocol for sites with multiple host races:

Materials:

We supply:

1. This protocol
2. 50 tea bags
3. 1 gallon ziploc with 250 g of silica desiccant beads (or multiple bags if you have told us you plan to collect from multiple sites)
4. 1 return label
5. 10 2 mL microcentrifuge tubes with 95%+ ethanol (for collection of dissected out insects)

You supply:

1. Sharpie/permanent marker
2. Clippers or scissors for field
3. Packing tape for mailing the samples back in

Site selection and plant ID:

Sites should have high enough density of *Eurosta* galls (see bottom of page for diagram of Eurosta galls vs other goldenrod galls) to collect, measure, and dissect at least 50 (ideally 200) galls this Spring. Since we are particularly interested in the parasite and parasitoid genetics in multi host plant systems, it is ideal if sites have more than 200 galls so that galls can also be dissected in the Fall for insect collection. Sites should have low risk of mowing, so you can visit it this Fall and this Spring (and ideally again in spring 2025, but that is flexible).

To determine the species of goldenrod at your site, reference this key
https://gobotany.nativeplanttrust.org/genus/solidago/

Leaf collection:

1. Sample a minimum of 20 stems (30 is even better) from each species. Make sure these selected stems have *Eurosta* galls growing on them, so we can be sure they are Eurosta's host plants.
2. Sampled stems should be spaced at least 2 meters apart to reduce the risk repeated sampling from clones
3. From each stem, collect 5 fresh leaves ideally without herbivore damage (see diagram at bottom of protocol to differentiate between gall types) and place the leaves from each stem in a separate tea bag
4. Put tea bags into gallon ziploc bag with silica gel. Please put leaf samples from different host plants in different ziploc bags.
5. Label the ziploc bag with host plant species, Site ID, and date. For 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 dissection:

For sites and regions with only non-*S. Altissima* plants or sympatric sites with multiple host plants, we are still interested!

We are particularly interested in comparing host-associated genetic differentiation of *Eurosta* across the speciation spectrum (e.g. host races vs. species) and parallelism of genetic differentiation across different geographical regions (e.g. genetic differentiation between host race *Eurosta solidaginis* on *S. altissima* vs. *E. solidaginis* on *S. gigantea* in Mid-west vs. New England). More distantly related *Eurosta* species can also serve as an outgroup in our genomic analysis. If we can get genetic information from their parasitoids, we can even test for co-speciation patterns between *Eurosta* and its associated parasitoids. Therefore, although fall gall dissection is an optional extension, it is particularly valuable from a site like yours!

So if you have the time and the site has sufficient galls (at least 100 (ideally 250) *S. altissima* galls or 10 for non *S. altissima* in present), we would love if you dissected galls and sent us the insects

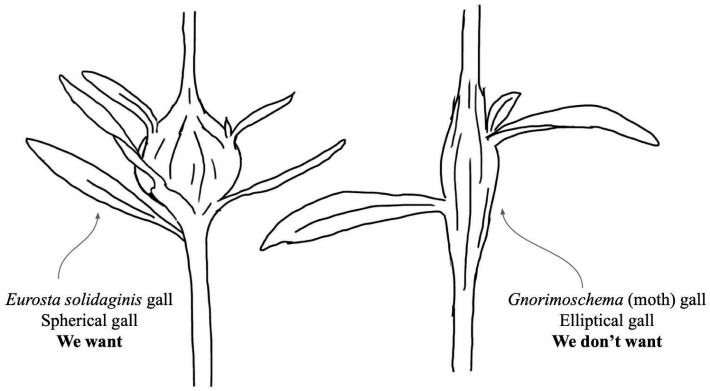
1. For S. altissima galls, collect a few (5-30) galls. It will be great if you can also measure the gall diameters (widest part of the sphere) for your collected galls. This will serve as a preliminary data for an NSF grant proposal this fall. Make sure to leave more than 50 (ideally 200) *S. altissima* galls for measurement and dissection in the Spring.
2. For galls on non *S. altissima* plants, even genetic information from a few individuals will be valuable by serving as an outgroup in the genomic analysis.
3. If you can get more individual insects (10+, ideally 30+), that's even better. We will have enough samples to get a good estimate of the population genomic parameters. The number of galls to collect to reach 10+ individuals depends on the local site (early death rates, parasitic rates). If you can, please collect at least 100 galls .
4. For *S. altissima galls* or non *S. altissima galls*, dissect them using clippers, scalpel, razor, or other sharp implement to reveal the inner chamber

5. Remove the insect (may be larva or pupa, we are interested in both) inside the gall and place in a microcentrifuge tube (1.5 or 2 mL) with 95% ethanol. We are currently working on a gall dissection primer which we will share when ready, but don't worry now about species ID. We will do that. Please keep in mind to put insects from different host plants into separate tubes and label them.
6. 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.
7. Place this bag into the shipping envelope.

Sending samples back:

1. Fill out and submit sample submission form https://forms.gle/3nUdLsZXQrFpKVRX9 (also linked in your kit confirmation email).
2. Tape prepaid return label over label on envelope used to send kit and drop off at FedEx or UPS
3. Expect an email letting you know when we've received your samples
4. 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.