This is a compilation of three protocols.

Gall Collection, Dissection and Data Protocol: page 2-9

Insect Emergence Phenology Protocol: page 10-11

Gall Growth and Plant Phenology Monitoring Protocol: page 12-16

For participants, **gall collection is mandatory**, while gall dissection, monitoring insect and plant phenology is optional. If you are unsure about identifying gall inhabitants, please send the collected galls to the following address:

Linyi Zhang
800 22nd St. NW
Suite 6000
Washington, DC 20052

Gall Collection, Dissection and Data Protocol

List of materials you need:

- Garden pruning shears for clipping galls
- Paper/cloth bag for containing galls
- Paper, permanent marker, pencils
- Drafting template with circles graduated in 1 mm increments (purchase link: https://www.amazon.com/gp/product/B077RTHQ8K/ref=ppx_yo_dt_b_search_asin_title?ie=UTF8&th=1).
- Plastic cups or paper plates
- 2 ml Eppendorf vials/2 ml glass vials (screw capped preferred, snap cap OK).
- Label tape
- 95% Ethyl alcohol
- Parafilm
- Shipping box

Scouting Galls

Identify collection sites in the autumn. After goldenrod dies back for the winter, it becomes very difficult to identify to species. Be sure to survey the sites before first frost to determine which species is being galled. You have been sent a leaf collection kit for molecular verification of plant ID.

The gall fly is a good disperser, so any two discrete patches of goldenrod separated by less than ~50 meters can be considered as in the population. If you find clusters of patches separated by >100 meters, treat them as different populations, and keep track of their lat-long coordinates.

To search for galls, you can use records of *Eurosta* on iNaturalist (www.inaturalist.org) to get an idea of where they might be found. However, keep in mind that iNaturalist records often skew toward more urban areas or parks with high visitor traffic. Alternatively, you can start by locating the fly's host plant, *Solidago altissima* (tall goldenrod), in their preferred habitats. This plant thrives in open lands with drier soils, such as prairies, grasslands, old fields, roadsides, savannas, woodlands, and forest openings. **In the past, we've had the most success in wildlife management areas, along roadsides, and near railroads.** The plants are most visible during their flowering season, from August to November, depending on the region (plants flower later at lower latitudes). Ideally, you should collect from natural preserves or protected areas, but roadsides with good parking access can also be suitable.

After identifying a suitable field site, be sure to clear out all the old galls by the end of the summer (sometime in August). These old galls are usually silver in color, have emergence holes, and are attached to dry, old stems. Removing old galls from the field is crucial to minimize the risk of mixing old with the current season's new galls when collecting the following spring. Be aware that

these sites might be mowed during the fall. To reduce this risk, it's advisable to contact local personnel or set up signs as a precaution.

Collecting Galls

Materials--

- Garden pruning shears for clipping galls
- Paper/cloth bag for containing galls
- Paper and permanent marker for labeling sampling sites

Aim for sites where you can collect 200+ galls. We need a random sample to estimate the size distribution, but also a large enough sample to include enough galls on the tails of the distribution to allow robust estimates for fitness function parameters. If you cannot locate a site with 200+ galls, a collection with as few as 50 still contributes useful information. Several scattered populations with a total sample size of 200+ is fine but keep track of their lat-long coordinates. If you find sites with thousands of galls, a sample size 400 will be fine; larger sample sizes will give diminishing returns on confidence intervals for the parameters of interest.

Collect the galls in mid-Spring (early-March in the far south, late-March to early April to the far north). You want to collect after Woodpecker attack is over for the winter, that is, after the birds start back foraging full time in the woodlands. But you also need to make sure you collect before the insects within the gall start to emerge.

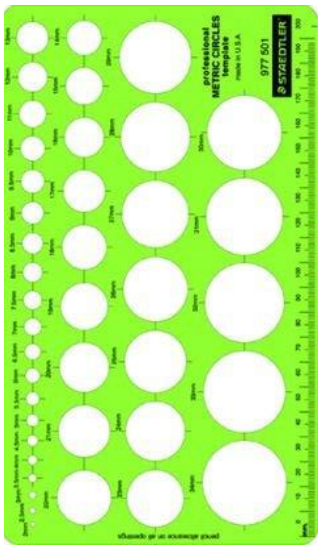
When collecting, snap the gall off the stem and place into a paper/cloth bag. If there is a time lag between collecting and processing, keep the galls in a cool place to prevent premature insect emergence. Galls kept in a fridge will last about a month before larvae dehydrate, making them more difficult to identify.

Measuring and Sorting by Size

Once the galls are collected, measure diameters. The following procedure is quick and resistant to errors.

Materials--

- Drafting template, with circles graduated in 1 mm increments (see photo; you can purchase it from here:https://www.amazon.com/gp/product/B077RTHQ8K/ref=ppx_yo_dt_b_search_asin_title?ie=UTF8&th=1).
- 28 containers, such as plastic beer cups or paper plates, numbered from 6 to 34, corresponding to the typically smallest and largest diameter classes (you supply).
- Data sheet(s), downloaded from https://continentalgallproject.github.io/website/.



Procedure—

- Pass each gall through the template holes. It's size is the smallest hole diameter that it can pass through without forcing.
- Place the gall into the container corresponding to its size class.
- Continue until all galls are measured. Count the number in each category and indicate on Data Sheet

Dissecting, Identifying,* and *preserving Content

Dissect and inspect the content of each gall. PROCESS ONE SIZE CATEGORY AT A TIME, as it minimizes opportunities for mistakes.

Materials--

- Ordinary garden pruning shears (you supply).

Opening the gall—

- Use ordinary garden pruning shears (you supply).
- Open the gall by cutting partway in, longitudinally (from pole to pole). Use the blade to cut nearly half-way through, then twist to pry it open and reveal the gall's central chamber, where the larvae reside.
- DO NOT cut all the way through – you will destroy the larva inside.
- DO NOT cut crosswise through the gall's equator – it will be difficult to pry open without also cutting through the central chamber and thus destroying the larva inside.

Identification and recording occupant—

- Using the identification key below, determine who occupies the gall, and enter the corresponding code to the appropriate column on the data sheet (see example below)
- Occupant codes are:

  EU — *Eurosta solidaginis*, the gallmaker
  gig — *Eurytoma gigantea,* external parasitoid wasp
  obt — *Eurytoma obtusiventris,* internal parasitoid wasp
  mc — *Mordelestena convicta*, inquiline beetle

wp — gall empty, pecked open by downy woodpecker
ck — gall empty, pecked open by black-capped chickadee
eld — gall empty, Early Larval Death, no sign of occupancy
uk — gall empty, but signs of occupancy/attack by another insect (i.e., frass)
eum — presence of both *Eurosta solidaginis*, the gallmaker and the inquiline beetle
gigm — presence of both *Eurosta gigantea* and the inquiline beetle
obtm — presence of both *Eurytoma obtusiventris* and the inquiline beetle
wpm — presence of both woodpecker predation and the inquiline beetle.
ckm — presence of both chickadee predation and the inquiline beetle.

- Tally the number of each occupant type for each size class at the bottom of the data sheet. Double check to make sure the numbers add up to the total.
- Transcribe tally to the excel sheet linked on the website.
- To upload your tally data file, fill out and submit the google form linked on the website, following prompts to enter host plant, latitude & longitude, and other info on the collection site and local investigators.
- Enter the data into the excel file provided and upload it along with a scan of the original data sheet to the website https://continentalgallproject.github.io/website/. You can also send an email with these files to gall.size@utoronto.ca with subject line "*completed data sheet*"

Securing, Preserving, and Shipping Specimens

When performing the Dissections, preserve the larvae you recover from the galls, as sorted by species (EU, gig, bt, mc).

Materials--

- 2 ml Eppendorf vials/ 2 ml glass vials (screw capped preferred, snap cap OK; you supply)
- Label tape
- Paper and pencils
- 95% Ethyl alcohol (you supply)
- Parafilm (you supply)
- Shipping box (you supply)

Procedure--

- Put the live specimens of each type from each size class into separate vials.
- Label the vial on the side with site code, gall size, and year. (e.g., KGR:09: 24).
- Cut a small piece of paper that would fit inside a vial and use a pencil to write down the same labeling info, put that piece of paper inside the vial.
- Wrap labeled vial tightly in parafilm.
- Pack in shipping box so that samples remain upright.
- Send by courier to the Zhang lab at George Washington Univ., using the address at the front of the document and on the project website (continentalgallproject.github.io/website/). Email the tracking number to o.bronzomunich@gwu.edu and gall.size@utoronto.ca to let us know to expect a shipment.

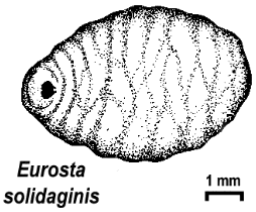
NOTE: groups that cannot participate in Project 1 can still contribute specimens for the genetic analysis. So, if gall numbers in your area are too low to get a sample of >50, you can nonetheless send a smaller number of specimens for sequencing analysis in support of this effort.

Upload the table to the website, following prompts to enter host plant, latitude & longitude, and other info on the collection site and local investigators.

Identification Guide for Gall Occupants

EU — *Eurosta solidaginis*, the gallmaker

A cream-colored, ovoid larva, without distinct head capsule, but with black, anteriorly-directed mouth hooks. Gall central chamber ovoid, and lined with compacted, gray frass. Exit tunnel extending to just below the gall epidermis often seen.



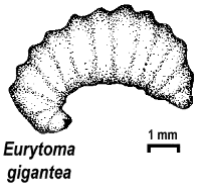
Larva

Pupae

Adult female

gig — *Eurytoma gigantea,* external parasitoid wasp

A white-colored, drop-shaped larva, with head capsule, brown mandibles, and tapered posterior end. Variable in size (Females ~5 mm long, males ~2 mm). Gall inner chamber irregular, often with brownish frass.

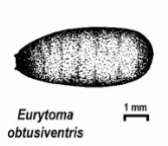


Larva

Adult female

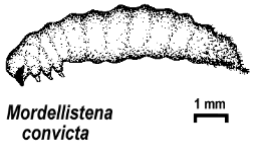
obt — *Eurytoma obtusiventris,* internal parasitoid wasp

A brownish, ovoid pupal case, ~3-5 mm long. In the fall and winter, they are already in the cocoon mode.



mc —, inquiline beetle

Cylindrical larva; ~4-5 mm long, < 1 mm wide; three legs; hairs on posterior segments; galls have irregular brown tunnels through pithy region; larva usually in tunnels.

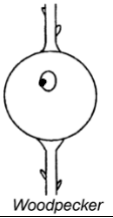


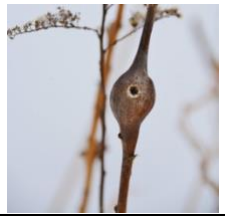
Larva

Adult

wp — *Pocoides pubesneces*, Downy Woodpecker

Round, nearly cylindrical hole, ~3mm diameter, chiselled from surface into central chamber of an empty gall.





ck — *Poecile atricapillus*, Black-capped Chickadee

Irregular, broadly conical hole ripped from surface into central chamber of an empty gall.

eld — Early Larval Death

Typically, 15-30% of gallmakers die after gall initiation but before maturation. They leave galls without a discernable central chamber, although there may be voids in the surrounding tissue due to shrinkage from dehydration. Occasionally these galls contain small drops of resin, but little or no frass.

uk — unknown

A small percentage of galls may have no inhabitants but contain frass or show signs of tunneling. Most of these are probably an instance in which *Mordelestena* has burrowed out of the gall and into the stem.

Data Sheet Example

This format is the least prone to errors when recording observations.

First measure and sort galls by size class into labeled containers (e.g., beer cups, paper plates). Then tally the number of galls per size class in the TOTAL row of the table.

Next, working with one size class at time, crack open each gall and record the occupant, recording the result for each gall is the appropriate cell. Tally the counts, by size and occupant, in the table and make sure they match the TOTAL row.

Transcribe and upload data to the website, following the directions given above

	Gall Diameter																												
frequency	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
24																													
23																													
22																													
21																													
20														EU															
19														EU															
18													EU	EU															
17													EU	mc															
16													gig	EU	EU														
15													wp	mc	mc														
14												mc	mc	eld	obt														
13												EU	EU	EU	EU		EU												
12												mc	EU	wp	EU		obt												
11												EU	mc	EU	wp	EU	wp												
10												gig	gig	ck	eld	obt	ck												
9							eld					obt	obt	gig	EU	EU	EU												
8							EU				gig	obt	EU	EU	obt	wp	mc	wp											
7							gig	gig			mc	eld	eld	obt	mc	wp	mc	mc											
6						gig	EU	gig		EU	gig	EU	EU	EU	EU	obt	obt	mc	wp										
5						mc	gig	obt		eld	mc	obt	gig	obt	obt	wp	wp	wp	ck										
4						gig	gig	mc	eld	mc	eld	mc	mc	eld	wp	EU	EU	obt	mc										
3					eld	eld	eld	EU	mc	mc	obt	EU	mc	EU	mc	mc	mc	EU	wp										
2			gig		obt	gig	obt	eld	gig	gig	gig	UK	eld	obt	wp	mc	obt	mc	wp		wp								
1		eld	mc		gig	EU	eld	eld	gig	gig	obt	mc	EU	EU	EU	mc	wp	wp	mc		mc								
Eurosta- ***EU***	0	0	0	0	0	1	2	1	1	1	0	4	7	10	6	3	2	1	0	0	0	0	0	0	0	0	0	0	0
E.gigantea- ***gig***	0	0	1	0	1	3	3	2	1	2	3	1	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E.obtusiventris-- ***obt***	0	0	0	0	1	0	1	1	0	0	2	3	1	3	3	2	3	1	0	0	0	0	0	0	0	0	0	0	0
M.convicta-- ***mc***	0	0	1	0	0	1	0	1	1	2	2	4	4	2	3	3	2	3	2	0	1	0	0	0	0	0	0	0	0
Woodpecker-**wp**	0	0	0	0	0	0	0	0	0	0	0	0	1	1	3	3	3	3	3	0	1	0	0	0	0	0	0	0	0
Chickadee-**ck**	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
Early Lar Death--**eld**	0	1	0	0	1	1	3	2	1	1	1	1	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unkown--**uk**	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL	0	1	2	0	3	6	9	7	4	6	8	14	18	20	16	11	11	8	6	0	2	0	0	0	0	0	0	0	0
Hostplant =	*S. altissima*						LAT =		*44.013053*				LON =		*-79.321744*							Participant name=					*Wilbur*		

Scan and email the original data sheets to gall.size@utoronto.ca with subject line "*completed data sheet*"

Insect Emergence Phenology Protocol

Monitoring Emergence

Simply, you will cage a collection of galls, then capture the insects as they emerge over the course of Spring. ***<u>It is important that these individuals experience the natural pace of spring warming at your latitude</u>*.** Therefore, keep the emergence cage in an outdoors situation. A building roof or home porch or is fine.

Materials—

- 100-150 galls collected from your local populations in the mid-late Spring (***in addition*** to those collected for gall dissection protocol above; need not be from the same site)
- Insect Emergence Cage (purchase link: https://www.amazon.com/dp/B07CWRYTLH/ref=sspa_dk_detail_0?pd_rd_i=B07CWRYTLH&pd_rd_w=U1lb2&content-id=amzn1.sym.f2f1cf8f-cab4-44dc-82ba-0ca811fb90cc&pf_rd_p=f2f1cf8f-cab4-44dc-82ba-0ca811fb90cc&pf_rd_r=G4W3DZZ7G16MYZQYKDFX&pd_rd_wg=xylLK&pd_rd_r=54d9e4d0-320b-42f6-b94c-dcb937420f65&s=toys-and-games&sp_csd=d2lkZ2V0TmFtZT1zcF9kZXRhaWxfdGhlbWF0aWM&th=1)
- Insect aspirator
- Eppendorf vials (screw capped preferred, snap cap OK)
- Label tape and pencil
- 95% Ethyl alcohol
- Parafilm
- Shipping box

Emergence cage

Proceedures—

- Ensure that the galls do not become dehydrated or moldy. Place the cage on a bench top where excess water can drain off and spray galls in cages from all angles every morning and again in the late afternoon. Each time you spray the galls down, water should start dripping off of the galls. If fungus-infected galls were separated out and placed in a bag, do not spray these bags, just check this bag for fly emergence.
- Check the cage for emerged, adult insects at least three times per week. It is easy to miss flies if you just check unopened bag/cage.
- Aspirate insects into vials. Make sure only one species per vial.
- Label each vial with site code, species code emergence date, and year (e.g., KSR:obt:15Jun:24), using a marker that will not fade if alcohol leaks.
- Record the number of emergents per species per emergence date on the data sheet downloaded from the website (see below).
- Wrap vials in parafilm pack into shipping box and send by courier to the Zhang lab at George Washington Univ., using the address on the project website.
- Transcribe data to the excel sheet linked on the website.
- To upload your data file, fill out and submit the google form linked on the website, following prompts to enter host plant, latitude & longitude, and other info on the collection site and local investigators.
- Scan and email the original data sheets to gall.size@utoronto.ca with subject line "*completed data sheet*"

Data Sheet Example

The datasheet will have columns for site, plant, date, and emerging species. Each row corresponds to date the insects were retrieved from the cage.

Transcribe and upload the data following directions above.

Site	Plant	Date	Eurosta	E. obt	E. gig female	E. gig male	M. convicta	other
KSR	S. alt	15-May	1	0	0	0	0	0
KSR	S. alt	18-May	3	1	0	0	0	0
KSR	S. alt	21-May	8	1	0	0	0	0
KSR	S. alt	24-May	4	2	0	0	0	0
KSR	S. alt	27-May	1	0	0	0	0	0
KSR	S. alt	30-May	0	1	0	0	0	0
KSR	S. alt	01-Jun	1	0	0	0	0	0
KSR	S. alt	04-Jun	0	0	0	0	0	0
KSR	S. alt	07-Jun	0	0	0	0	0	0
KSR	S. alt	10-Jun	0	0	0	0	1	0
KSR	S. alt	13-Jun	0	0	0	0	5	0
KSR	S. alt	17-Jun	0	0	0	0	2	0
KSR	S. alt	20-Jun	0	0	1	0	1	1
KSR	S. alt	23-Jun	0	0	3	1	0	0
KSR	S. alt	27-Jun	0	0	2	1	0	0

Gall Growth and Plant Phenology Monitoring Protocol

Here you will be concerned with the phenology of the gall itself and that of its hostplant.

Monitoring initiation and growth of gall, and flowering phenology of plant

Participants will monitor plant population for gall initiation. The basic procedure is to visit a population site weekly from late-spring to mid-summer. On each visit, mark newly-formed galls, then measure their dimeter. Then, once it is marked, each gall is re-measured (diameter) on subsequent visits, up to late July, when gall reach their mature size (see the growth curve figure above). Starting in August, revisit plants bi-weekly and record their flowering stage.

Materials—

- Surveyors flagging tape (you supply)
- Dial calipers (you supply)
- Clip board for data sheet (you supply)
- Datasheet, downloaded form website.

Proceedure--

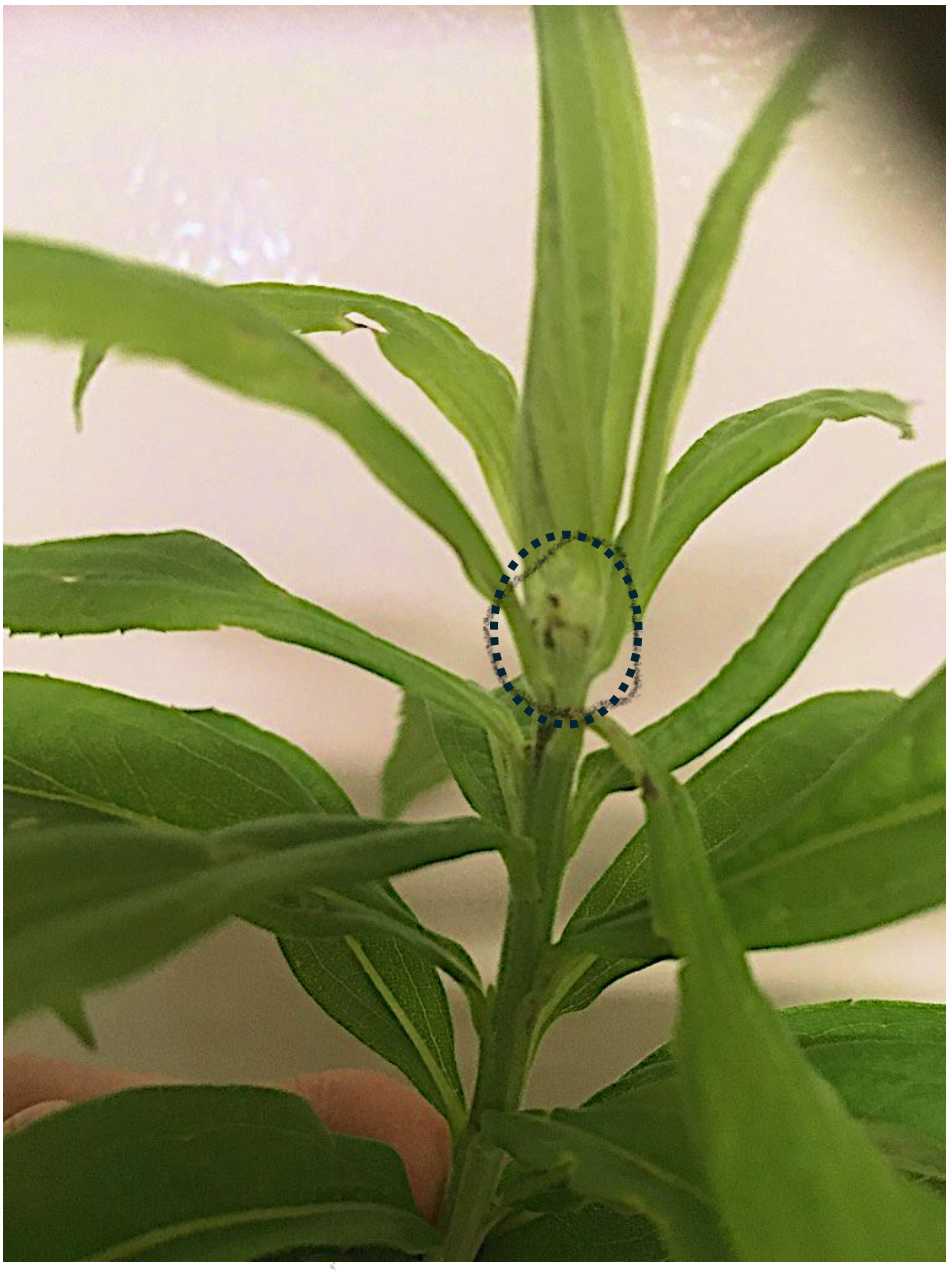
- Establish a path through the field site, which you will re-walk weekly.
- In early spring, mark stems along the path with newly forming galls (see below) with number flagging tape labeled with an ID number, 1 to N.
- Record date for each new gall on data sheet (see below).
- On subsequent visits, record diameter of every marked gall. Repeat until galls reach the size plateau, typically in mid-July (see the growth curve figure above). By the end you will have somewhere between 5 and 10 repeated measurements per gall.
- Starring in August, visit fields bi-weekly, and record the flowering stage for each marked plant, using the ranking scheme described below.
- In December, collect the labeled galls, measure their final diameter, dissect out and identify occupants, using the information above.
- Transcribe data to the excel sheet linked on the website.
- To upload your data file, fill out and submit the google form linked on the website, following prompts to enter host plant, latitude & longitude, and other info on the collection site and local investigators.
- Scan and email the original data sheets to gall.size@utoronto.ca with subject line "*completed data sheet*"

Tips on identifying newly-formed galls

The new galls will appear as slight swellings at the base of the plant's terminal bud (see photo). They are slightly paler than the stem below and the bud above. You can verify by touch. Gently pinch the base of the bud. A new gall feels larger and more solid than the base of an ungalled bud. You may also see a series of pin holes on the leaves just below the new gall. These are the leaves that wrapped the bud back when the gallfly deposited the egg; the holes are the

oviposition punctures. If you have doubts, mark the stem anyway; if it is a "false positive' you can exclude it later. Note that some of these "false positives" will be galls induced by the Goldenrod Bunch Gall Midge (*Rhopalomyia solidaginis*). As the bunch galls develop in the bud they will very obviously not be ball galls on the stem.

As the spring progresses, you may encounter galls along the transects that you missed on previous dates…this always happens. Do not include these in the sample.



Newly-formed Gall

Note—the brown spots seen in this photo are not typical

Scoring Plant Flowering Phenology

As the summer comes to an end, field sites should be revisited bi-weekly to score the hostplant's flowering phenology. Please start before you see the first plant in flower to avoid truncated data. The most practical way to score phenology for this project is to use this scale, from 0 to 4.

0 → Terminal bud in vegatative state, no visible flower buds

1 → Flower buds visible, but not open

2 → From 1 to 25% of flower buds opened

3 → **>25% of flowers buds opened**

4 → **At least some flowers senescing**

Stie	Host	GallNum	IntDate	FinalDiam	Occupant	27-May	03-Jun	10-Jun	17-Jun	24-Jun	01-Jul	08-Jul	15-Jul	22-Jul	29-Jul	05-Aug	19-Aug	02-Sep	16-Sep	30-Sep	7-Oct
KRS	S.alt	1	27-May	25.3	Eu	4.9	9.8	16.8	22.4	24.7	24.9	24.8	24.9			0	0	0	2	3	4
KRS	S.alt	2	03-Jun	24.1	Eu	na	5.0	10.1	17.5	22.5	23.2	24.0	23.9			0	0	1	2	3	4
KRS	S.alt	3	03-Jun	23.3	mc	na	5.1	9.9	18.2	23.2	23.6	23.2	23.3			0	0	1	3	4	4
KRS	S.alt	4	03-Jun	20.0	obt	na	4.9	9.7	16.7	19.9	20.1	20.2	20.1			0	0	1	2	2	3
KRS	S.alt	5	03-Jun	24.1	Eu	na	5.5	12.0	19.0	23.3	23.8	23.6	23.6			0	1	1	2	4	4
KRS	S.alt	6	03-Jun	19.1	mc	na	5.3	11.5	17.5	18.0	19.0	18.9	19.0			0	0	1	2	2	2
KRS	S.alt	7	03-Jun	18.3	obt	na	5.3	10.9	16.8	17.2	18.5	18.5	18.4			0	0	1	2	2	2
KRS	S.alt	8	10-Jun	17.6	gig	na	na	5.0	10.0	14.3	17.1	17.2	17.1			0	0	1	2	2	3
KRS	S.alt	9	10-Jun	17.6	eld	na	na	4.9	9.9	13.2	16.1	17.3	17.2			0	1	1	3	4	4
KRS	S.alt	10	10-Jun	17.1	gig	na	na	5.9	12.0	14.1	15.9	16.9	17.0			0	0	0	2	2	3
KRS	S.alt	11	17-Jun	15.9	gig	na	na	na	5.4	8.1	11.0	15.1	16.0			0	0	1	2	2	3
KRS	S.alt	12	17-Jun	14.8	eld	na	na	na	5.2	8.3	10.1	14.9	15.1			0	0	1	2	3	4

Gall ID information;

Initiation date, final size, content

Gall diameter over time (optional)

Flowering Phenology Score (optional)