



HERBVAR

Project Manual & Field Protocols

Prepared by
The HerbVar Steering Committee

HerbVar Project Manual & Field Protocols

The Herbvar Steering Committee

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Preamble

This book is a manual for researchers involved in the the [HerbVar Project](#). It includes a checklist for new collaborators, guidelines on accessing, adding, and using project data, tutorials for using the RStudio Project Templates for conducting analyses and preparing manuscripts, protocols for field work, and guides for administering the HerbVar Network resources (e.g., website, data portal).

The HerbVar Steering Committee would like to express their appreciation to the following authors of *Alternative & Protocols* and Guides to Data Collection:

- *Reproductive Damage*: Susan Whitehead (Virginia Tech), Phil Hahn (University of Florida), Paul Ode (Colorado State University), Will Wetzel (Montana State University)
- *Cacti & Succulents*: Judie Bronstein (University of Arizona), Victoria Luizzi (University of Arizona)
- *Trees*: Bastien Castagnéryrol (NRAE-UMR BIOGECO), Amy Trowbridge (University of Wisconsin, Madison), Will Wetzel (Montana State University), Moria Robinson (Utah State University)
- *Estimating Damage*: Ian Pearse (U.S. Geological Survey), Zoe Getman-Pickering (University of Massachusetts, Amherst), Julie Davis (Cornell University), Bastien Castagnéryrol (NRAE-UMR BIOGECO), Will Wetzel (Montana State University)
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This guide is a [Quarto Book](#) hosted on the [HerbVar Network's Github site](#). Any team member can edit the text, add new sections, or make suggestions for improvement either by [pull request](#) or by [posting an issue](#) on the HerbVar Manual's repository. For more information and tutorials for doing so, see Chapter [26](#).

Part I.

What is HerbVar?

1. HerbVar Overview

Welcome to HerbVar! We are a collaborative network of researchers interested in understanding how plant–herbivore interactions vary across plant species around the globe. Most of the field’s current understanding of large-scale patterns in herbivory and plant–herbivore interactions come from studies focused on average levels of herbivory. We aim to advance our fundamental understanding of plant–herbivore interactions by quantifying variation in patterns of plant–herbivore interactions within and across sites.

1.1. Project Goals

- To gather standardized data on the distribution of herbivory on individual plants within populations for species and sites across the globe.
- To promote the use of HerbVar data to generate testable hypotheses and publications that advance our fundamental and applied understanding of the role of variability and distributions in plant–herbivore interactions.
- To develop theory and statistical tools for studying the role of distributions in plant–herbivore interactions.
- To engage researchers globally and across career stages and backgrounds in collaborative science.
- To increase awareness of and education about the importance of variability in biological processes at graduate and undergraduate levels.

1.2. Motivation

Published studies and personal observations suggest the distribution of herbivore feeding damage among individual plants within a population is often highly skewed such that most plants and plant parts (leaves, reproductive structures, etc.) experience relatively low levels of damage, and a small fraction of plants experience disproportionately high levels of damage. Theory suggests that such variability can have dramatic ecological and evolutionary consequences. For example, variability among plants can lead overall herbivore population size to be greater or less than expected based on average plant quality and asymmetric fitness surfaces can lead to over-investment in defensive traits. *Surprisingly, despite the established theoretical importance and potential generality of variability in herbivory, it has received little empirical attention, limiting our fundamental understanding of how plants and herbivores interact.*

1.3. Getting Involved

All professionally-trained researchers with expertise in plant-herbivore interactions are welcome to join HerbVar as a member. We ask members to read this manual before reaching out about joining. Once you have decided to join, please email [Will Wetzel](#) to get the process started. **Members can get involved in the network in four main ways:**

1. **Become a Site PI** by collecting data using one of the HerbVar Protocols described in this manual. Submit data towards a sampling objective and become a co-author on a publication resulting from that sampling objective (see Chapter 5 and Chapter 7).
2. **Propose a new Working Group.** Working Groups analyze and publish existing data, design and coordinate new add-on data collection efforts, and develop educational resources with HerbVar data (see Section 6.1).
3. **Join an existing Working Group** open to new members. See the [Working Groups page of our website](#) for a list of our Working Groups.
4. **Propose a new add-on data collection effort** to have Site PIs collect data you would like to analyze and publish (see Chapter 9).

This manual explains the process for each of these ways of getting involved.

1.4. Questions?

More information on the HerbVar Project is available on the [project website](#). If you can't find the information you're looking for in this manual or the website, please contact one of the HerbVar PIs or a member of the HerbVar Steering Committee (see Chapter 2 for their contact information).

2. Network Structure

We are a collaborative and inclusive group of researchers who are working together to advance our understanding of plant–herbivore interactions. Membership in HerbVar is determined by participation in data collection and/or significant managerial work, writing or analysis.

2.1. Network PIs

HerbVar is led by the Network PIs:

- [Will Wetzel](#), Montana State University
- [Nora Underwood](#), Florida State University
- [Brian Inouye](#), Florida State University
- [Susan Whitehead](#), Virginia Tech, and
- [Phil Hahn](#), University of Florida

2.2. Steering Committee

The HerbVar Steering Committee guides the development and implementation of HerbVar policy with input from HerbVar members as needed. This includes the organization of the network, participation guidelines, authorship guidelines and protocols, review of proposals for papers and expanded data collection, and curation of HerbVar data and GitHub tools for working with the data. The Steering Committee is also responsible for producing core papers using the HerbVar data and involving all relevant Site PIs that meet Authorship Guidelines. The members of the Steering Committee are:

- Nora Underwood nunderwood@bio.fsu.edu
- Brian Inouye binouye@bio.fsu.edu
- Susan Whitehead swhitehead@vt.edu
- Phil Hahn hahnp@ufl.edu

2. Network Structure

- Lee Dyer nolaclimber@gmail.com
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- Ivalu Cacho ivalu.cacho@gmail.com
- Karen Abbott kcabott@case.edu
- Will Wetzel wiliam.wetzel@montana.edu

2.3. Members

HerbVar membership is open to all professionally-trained researchers with expertise in plant-herbivore interactions who plan to collect data or participate in a working group. Members can become Site PIs, can join or lead working groups, or both (see below and Chapter 1).

2.4. Site PIs

Data are collected and contributed by Site PIs and their collaborators or colleagues. Site PIs earn authorship on core papers according to the Authorship Guidelines (see Chapter 7).

2.5. Working Groups

Specific HerbVar goals, projects, and papers are pursued by Working Groups. New working groups will be formed after review and approval by the HerbVar Steering Committee, who will consider their goals and plans for leadership and member recruitment. All HerbVar members are invited to propose working groups for papers based on HerbVar data and/or new types of network data collection. The proposal process typically involves emailing a one page description of the project, including goals, methods, and plans for running the group, to the Steering Committee. For more details, see Section 6.2, Section 3.1, Chapter 9, and Chapter 7. Approved working groups will have the support of the network including access to relevant unpublished data, use of pre-written R code for wrangling and cleaning HerbVar data, use of the HerbVar GitHub organization, and publicity for completed work. [Here is our current list of Working Groups.](#)

3. HerbVar Datasets

3.1. Core HerbVar Dataset (Phase 1)

The Phase 1 HerbVar dataset was begun in 2017 and closed for contributions prior to submission of our 2023 paper in *Science*. For the Phase 1 Core Dataset we prioritized surveys that added phylogenetic and geographic breadth to analyses - it includes estimates of herbivore damage on more than 50,000 plant individuals from 790 populations of 503 species in 135 families. The sampling occurred across 116 degrees of latitude and was conducted by 127 research teams working in 34 countries.

The first paper that made use of this dataset was published in *Science* and can be read [here](#). The subset of the Core Data that was used in the paper is [archived and available at Dryad](#).

Working groups are currently analyzing parts of the Core Dataset that were not used for the *Science* paper, and HerbVar members are welcome to propose additional analyses using unpublished portions of the Core Dataset. The still-unpublished Core Data used in these analyses will be archived at Dryad when the resulting papers are published.

3.2. Ongoing Data Collection (Phase 2)

Add-on data collection projects have been added since the completion of Phase 1. We are currently prioritizing:

1. Collecting data to assess damage to plant reproductive structures (see Chapter 16),
2. Collecting data from species in our five focal plant families: Apocynaceae, Asteraceae, Fabaceae, Rubiaceae, and Solanaceae,
3. Collecting data from our three focal plant species: *Taraxacum officinale* (Asteraceae), *Plantago lanceolata* (Plantaginaceae), *Plantago major* (Plantaginaceae).

3.3. Proposing the Collection of New Datasets

HerbVar network members are also welcome to propose new add-on projects. See Chapter 9 for details on how to propose add-on projects.

4. Herbvar Products

4.1. Publications

A .bib file of all HerbVar presentations is available for download [here](#).

Galmán, A., P. G. Hahn, B. D. Inouye, et al. (2024). “Global test of the enemy release hypothesis reveals similar patterns of herbivory across native and non-native plants”. In: *bioRxiv*. DOI: <https://doi.org/10.1101/2024.10.21.619365>.

Pan, V. S. and W. C. Wetzel (2024). “Neutrality in plant–herbivore interactions”. In: Proceedings of the Royal Society B: Biological Sciences 291.2017, p. 20232687. [DOI: 10.1098/rspb.2023.2687](<https://royalsocietypublishing.org/doi/10.1098/rspb.2023.2687>).

The Herbivory Variability Network* †, M. L. Robinson, P. G. Hahn, et al. (2023). “Plant size, latitude, and phylogeny explain within-population variability in herbivory”. In: *Science* 382.6671, pp. 679-683. DOI: [10.1126/science.adh8830](https://doi.org/10.1126/science.adh8830).

Wetzel, W. C., B. D. Inouye, P. G. Hahn, et al. (2023). “Variability in Plant–Herbivore Interactions”. In: Annual Review of Ecology, Evolution, and Systematics 54.1, pp. 451-474. DOI: [10.1146/annurev-ecolsys-102221-045015](https://doi.org/10.1146/annurev-ecolsys-102221-045015).

HerbVar Steering Committee (2024). “HerbVar: Project Manual and Field protocols (v0.9.0)”. Zenodo. <https://doi.org/10.5281/zenodo.14232308>

4.2. Presentations

A .bib file of all HerbVar presentations is available for download [here](#).

Bruna, E. M. (2024). Github as a tool for promoting reproducibility and collaboration in the HerbVar Network. *HerbVar Steeting Committee Meeting*.

Galmán, A., P. G. Hahn, B. D. Inouye, et al. (2024). Global analyses of enemy release indicate similar herbivory mean and variability across native and non-native plants regardless of environment and plant characteristics. *Annual Meeting of the Ecological Society of America*, Long Beach, California.

Galmán, A., P. G. Hahn, B. D. Inouye, et al. (2023). Plant diversity determines differences in plant-herbivory interactions in native and exotic plants. *Annual Meeting of the Ecological Society of America*, Portland, OR.

Galmán, A., W. C. Wetzel, N. Underwood, et al. (2024). Global analyses of Enemy Release Hypothesis and the effects of environment and plant growth form. *International Congress of Entomology*, Kyoto, Japan.

4. Herbvar Products

- Pan, V. S., E. Ghosh, P. Ode, et al. (2024). Spatially Heterogeneous Phytochemical Landscapes Increase Herbivore Performance by Enhancing Herbivore Foraging. *Annual Meeting of the Ecological Society of America*, Long Beach, California.
- Pan, V. S. and W. C. Wetzel (2022). A neutral model of herbivory: fundamental constraints on patterns and variation. *Annual Meeting of the Ecological Society of America*, Montreal, Canada.
- Robinson, M. L., K. C. Abbott, E. M. Bruna, et al. (2021). Plant apparency shapes the distribution of herbivory within and among plant individuals: Data from the HerbVar Network. *Annual Meeting of the Entomological Society of America*, Denver, CO.
- Wetzel, W. C. (2023). Macroecological and macroevolutionary patterns of variability in plant–herbivore interactions. *Advances in Mathematical Ecology*, Pittsburgh, PA.
- Wetzel, W. C., K. C. Abbott, E. M. Bruna, et al. (2021). Macroevolutionary and global patterns of intraspecific variability in herbivory: Data from the HerbVar Network. *Annual Meeting of the Entomological Society of America*, Denver, CO.
- Wetzel, W. C., M. L. Robinson, L. A. Dyer, et al. (2020). Variability is a pervasive feature of plant–herbivore interactions: Data from The Herbivory Variability Network. *Annual Meeting of the Ecological Society of America*, virtual meeting.
- Whitehead, S. R., K. C. Abbott, E. M. Bruna, et al. (2021). Multi-scale spatial variation in herbivore damage to plant reproductive organs: Data from the HerbVar Network. *Annual Meeting of the Entomological Society of America*, Denver, CO.
- Whitehead, S. R., C. Quintero, Y. Sasal, et al. (2024). From leaf to biome: multi-scale variability in plant-herbivore interactions. *Annual Meeting of the Ecological Society of America*, Long Beach, California.

4.3. Archived Datasets

- Wetzel, William; Hahn, Philip; Inouye, Brian et al. (2023). Plant size, latitude, and phylogeny explain within-population variability in herbivory [Dataset]. Dryad. <https://doi.org/10.5061/dryad.44j0zpckm>
- Pan, Vincent; Wetzel, William (2024). Neutrality in plant–herbivore interactions [Dataset]. Dryad. <https://doi.org/10.5061/dryad.qjq2bvqnz>

Part II.

Become a Collaborator

5. Organize an HerbVar Site

The HerbVar Site PI is one of the most important roles in the network. Site PIs establish research sites, collect and submit data to ongoing sampling objectives, and assist with analyses and manuscript editing. **Site PIs must adhere to the following guidelines:**

- Be professionally-trained researchers with expertise in plant-herbivore interactions.
- Be inclusive, collegial, and collaborative in their interactions with other members of HerbVar.
- Have or obtain supplies necessary for conducting their own surveys. As a distributed network, HerbVar does not have funding for data collection.
- Contribute data gathered using a standard HerbVar protocol or a modified protocol approved by the Steering Committee (see Chapter 14).
- Contribute species-level data on each species they survey (i.e., species-level traits), as requested by the Steering Committee.
- Recognize the contributions of all individuals involved in collecting HerbVar data, even if that individual's participation does not satisfy the criteria for authorship (see Chapter 7).
- Site PI's should provide names, positions, and institutional affiliations of individuals who substantially contribute to data collection with them ("additional participants" columns in 'HerbVar Coordination' and 'Completed Surveys' documents). These additional participants will be recognized via the HerbVar website and via a link in the acknowledgments of HerbVar publications; we also encourage Site PIs to recognize local participation".
- Follow the HerbVar data sharing and usage agreement (available in Section 23.1)
- Ensure that your data are high quality. Check your data for errors before submitting them, and inform us immediately of any errors that you discover after submission. We identify and fix data errors to the extent possible as part of our data wrangling process. We understand that some errors are a normal part of data collection, but datasets that have an unreasonable number of errors will be rejected and not count towards authorship. We will not count errors against you if you contact us about them. Some mistakes are expected; the most important thing is for Site PI's to be alert for errors and to communicate openly about them when they happen.

5.1. Standards for Authorship and the HerbVar Authorship Process

Authorship on papers arising from HerbVar data will be earned through significant contribution to the scientific process. Final authorship assignment is the responsibility of the lead author of the manuscript (where the lead author is the project leader approved by

5. Organize an HerbVar Site

the Steering Committee). *We strongly favor being inclusive in authorship.* The detailed authorship guidelines can be found in Chapter 7.

5.2. Site PIs and authorship on HerbVar papers

Site PIs who contribute to sampling objectives will earn authorship on the core paper that arises from that sampling effort. For more details on criteria for authorship see Chapter 7.

5.2.1. Authorship on “Core HerbVar Papers”

The HerbVar Network launched with a Steering Committee commitment to produce two papers using the core HerbVar data. Site PIs earn co-authorship on these papers by providing at least 3 surveys (though more are always welcome) to the core dataset.

5.2.2. Authorship on “add-on project” Papers

The Steering Committee has launched three add-on projects (“Focal Species”, “Focal Families”, “Reproductive Damage”); proposals for additional add-on projects are welcome (see Chapter 9). Site PIs earn co-authorship on one paper from each add-on project by contributing at least three surveys (or equivalent) that advance that project, in addition to the minimum three surveys required for Site PI status and authorship on the core HerbVar papers.

6. New Analyses with HerbVar Data

6.1. Overview

Our network is excited about researchers using HerbVar data for new analyses and publications. People interested in analyzing HerbVar data for publication should read Chapter 9 and Chapter 7 for a detailed outline of how to propose a project and the authorship guidelines.

In brief, the prospective author(s) look at the list of products already being worked on, submit a working title and abstract to the HerbVar Steering Committee, and receive feedback/approval. The proposed publication is then listed on the HerbVar site for other network members to see. Authors are encouraged, but not required, to invite additional network members to work with them as appropriate. See Authorship Guidelines (Chapter 7) for discussion of how and when to invite others. This process provides benefits such as access to unpublished data, information on potential overlap of the paper with other completed or ongoing analyses, advice about how to interpret the data, GitHub tools customized for analysis and publication of HerbVar data, and publicity for the publication through HerbVar channels.

Working Groups typically have six months to initiate work and two years from proposal to manuscript submission (or equivalent for non-manuscript projects). If manuscripts do not progress within those time frames the Steering Committee may make the data available to others interested in working on those questions.

HerbVar's Collaborative Ethos

Although people are free to work with HerbVar's publicly available data independently, we hope that they will choose to work collaboratively with members of the network.

6.2. Using Publicly Available HerbVar Data

As papers using HerbVar data are published, HerbVar data associated with those papers become publicly available. There are two ways to work with these data:

1. Non-members and members can work independently with the published data, provided that they cite the datasets. As a courtesy, we request that individuals using published HerbVar data alert the HerbVar Steering Committee so we can track HerbVar-related publications.

6. New Analyses with HerbVar Data

2. Members and non-members can work with our published data and write papers through the HerbVar network as an HerbVar-sponsored Working Group. See Chapter 7 for details on how to propose new paper ideas and Working Groups.

In short, potential authors share their idea for a product with the Steering Committee, potentially inviting some members to join them although not necessarily for papers that are best done by a small author group. In return, HerbVar provides information on potential overlap of the paper with other completed or ongoing analyses, advice about how to use and interpret our data, pre-written R code for data wrangling and cleaning (via our GitHub organization), and publicity for the publication through HerbVar channels. An additional benefit of working as an HerbVar-sponsored working group is access to unpublished data (see below).

6.2.1. Citing Published HerbVar Data

Please be sure any manuscripts or presentations include citations to the archived dataset and the original paper for which they were used, as well as an acknowledgement of NSF's funding of the HerbVar RCN, as described in Chapter 7.

6.3. Using Unpublished HerbVar Data

Unpublished data will be available to relevant HerbVar Working Groups as soon as they are compiled by the Steering Committee. Access will be provided through the [HerbVar GitHub organization](#). We encourage HerbVar Working Groups to use our data to publish papers and educational materials, but it is important that all products be conceptually distinct from ongoing projects. Therefore, please review the proposed add-ons and in-progress papers and read the HerbVar Authorship Guidelines, which describe the process for forming a working group (see Chapter 7).

6.3.1. Citing Unpublished HerbVar Data

No permission is required to use Herbvar Data for educational purposes (e.g., course assignments) or presentations. We do ask, however, that you (1) notify the Steering Committee so we can track HerbVar's impact and (2) acknowledge the data as follows: "Data were made available through the HerbVar Network, a project supported by the National Science Foundation (DEB-2203582 to W. Wetzel *et al.*)"

If you intend to eventually publish the material in which you are using unpublished HerbVar Data, you must follow the HerbVar Network Authorship Process (see Authorship Guidelines in Chapter 7). This process is designed to make sure contributors get credit for their data, to reduce overlap, to foster collaboration, to streamline use of HerbVar data to produce papers, and to help publicize HerbVar products. Chapter 7 also provides information on how to cite datasets and NSF support of the HerbVar RCN.

7. HerbVar Papers & Authorship

7.1. Overview and Philosophy

The primary goals of the HerbVar authorship process are to consistently, accurately, and transparently attribute the contribution of each author to a paper, to encourage participation in manuscripts by interested scientists, and to ensure that each author has made sufficient contribution to the paper to warrant authorship. Secondarily, authoring a paper within the HerbVar process benefits authors by reducing overlap among papers, fostering collaboration, streamlining use of HerbVar data to produce papers through GitHub tools, and helping to publicize HerbVar products. While prospective authors can use published HerbVar data to create products on their own, we encourage all authors working with HerbVar data to work with the network.

The HerbVar authorship process has been heavily influenced by the authorship process implemented by the [Nutrient Network](#). This process provides ground rules for a fair and inclusive process for establishing authorship while not diluting the value of authorship on a manuscript. HerbVar is grateful to be able to build on the foundation of NutNet's model for collaborative international ecology. The text below is edited to reflect HerbVar-specific policies, but some is drawn directly from the [NutNet authorship document](#). Differences reflect the fact that HerbVar is a much larger network (with hundreds of contributors) and a more fluid network (contributors might only contribute data once, rather than actively maintaining an experimental site).

7.2. Herbvar Network contributors

The following are definitions for the different types of network contributors:

- **Steering committee:** Responsible for overall network administration.
- **Site PI:** A contributor that has submitted at least three herbvar surveys for one of the main or add-on objectives. Note that a person can be a site PI for the main objective, but not necessarily a site PI for one or all of the the add-on projects, depending on the completed surveys.
- **Member:** Anyone who is currently or planning to work with the data, either through data collection, working group, etc.

7.3. Article Categories

In general, HerbVar aims to be as inclusive as possible. At the same time, we acknowledge that massively multi-authored papers (e.g. with all HerbVar members) present substantial challenges for lead authors and might discourage initiation of new projects. To balance these factors, there are two types of published products in the HerbVar process: “**All PI Papers**” and “**Working Group Papers**”.

7.3.1. “All-PI” Papers

All-PI Papers invite all site PIs meeting authorship requirements to be co-authors, acknowledging the fundamental importance of contributing data to HerbVar.

The HerbVar Network launched with a Steering Committee commitment to produce two papers using the core HerbVar data collected during the first phase of sampling. Site PIs earn co-authorship on these papers by providing at least 3 surveys (though more are always welcome) to the core dataset. The Steering Committee has so far launched three add-on projects (Focal Species, Focal Families, Reproductive Damage). Additional add-on projects may be created - see Datasets and Proposals for New Data Collection. Site PIs earn co-authorship on one paper from each add-on project by contributing at least three surveys that advance that project, in addition to the minimum three surveys required for Site PI status and authorship on the core HerbVar papers. See Section 9.1, [the HerbVar website](#) and our Species selection document (in the HerbVar Protocols folder) for information on our current sampling objectives and planned manuscripts. We will be flexible on the minimum number of surveys, particularly if substantial additional intellectual contributions are made to project development (e.g., creation of documents and protocols, recruitment of collaborators able to work in under-sampled regions or with under-sampled taxa, or other network coordination work). The Steering Committee will review instances that do not meet the minimum criteria on a case-by-case basis. Please reach out if you have questions or concerns. Collaborators who set out to complete 3+ surveys but are only able to contribute 1-2 surveys will be given the opportunity to maintain authorship by contributing in other ways. Potential contributions that could make up for missing surveys include but are not limited to working on our primary database, developing our traits and/or environmental databases, and/or collecting other metadata. In addition to contributing data, Site PIs who want to be co-authors must also contribute intellectually to at least one of the following: development of questions and overall study design, data analysis or interpretation, writing, or editing.

Site PIs who contribute data but do not meet the criteria for co-authorship (e.g., submitting too few surveys, deciding to not make up for missing surveys with additional contributions (see criteria for authorship below, etc.) have two options for their data:

1. The Site PI will not be a co-author, but they agree to let HerbVar use and publish the data they contributed. They will be recognized via the HerbVar member list. The HerbVar member list will serve to acknowledge all contributors (even if they didn’t meet co-authorship criteria), and will be associated with each manuscript.

7. HerbVar Papers & Authorship

2. The Site PI will not be a co-author, and they do not agree to let HerbVar use and publish their data. All their data will be removed from all HerbVar databases. HerbVar will not use or publish their data. They will be removed from the HerbVar member list.

7.3.2. “Working Group” Papers

Working Group Papers allow for smaller teams to assemble to write additional papers using HerbVar data, broadening the scope of our collective work without requiring every lead author to manage hundreds of contributors. Typically, working group papers will be centered around already published HerbVar data analyzed in a new way and potentially combined with some new data. HerbVar supports working group paper authors in being as inclusive as is practical, and in connecting with appropriate co-authors to build new collaborations. A complete guide to proposing and writing Working Group papers, along with criteria and expectations for co-authorship, can be found in Chapter 8.

7.4. Authorship Disputes

The steering committee works to ensure communication across projects to avoid overlap of manuscripts, and provides guidance on procedures and guidelines. Disputes over authorship and manuscripts should be brought to the Steering Committee (Chapter 2) for resolution.

7.5. Manuscript Preparation

Please refer to Chapter 10 for details on the HerbVar Research Workflow and guide to preparing reproducible manuscripts. Chapter 12 includes required information to include manuscripts, such as citations of Herbvar datasets and acknowledgement of NSF support.

8. Proposing ‘Working Group’ Papers

Working Group Papers allow for smaller teams to assemble to write additional papers using HerbVar data, broadening the scope of our collective work without requiring every lead author to manage hundreds of contributors. Typically, working group papers will be centered around already published HerbVar data analyzed in a new way and potentially combined with some new data. HerbVar supports working group paper authors in being as inclusive as is practical, and in connecting with appropriate co-authors to build new collaborations.

8.1. Working Group Proposal & Review Process

If an HerbVar member would like to lead an HerbVar working group paper (i.e., serve as “Lead Author”), they should follow the steps below.

1. *Read the authorship policies* and guidelines below.
2. *Consult the [Working Group page of the HerbVar website](#)* to review current and proposed *Working Group projects*, and contact the listed lead author on any similar proposal to minimize overlap or to join forces.
3. *Select an approach* for forming the working group and inviting co-authors.
4. *Prepare a manuscript proposal*, and email it to one of the members of the [HerbVar Steering Committee](#). Your proposal should list:
 - i. the lead author(s)
 - ii. any other known authors
 - iii. plans for recruiting additional authors, if any
 - iv. the title
 - v. the abstract
 - vi. the specific types of data that you wish to use
 - vii. A brief overview of the response and predictor variables (if appropriate), and
 - viii. a timeline for analysis and writing.

Proposals are reviewed by the Steering Committee to ensure there is sufficient distinction from proposed and ongoing HerbVar papers. The Steering Committee may suggest altering or combining analyses and papers to resolve issues of overlap. *After receiving Steering Committee Feedback, your proposal will be posted on the HerbVar website.*

8.2. Following Approval: Research & Writing

Lead Authors should start work on the project within six months of the approval of their project proposal by the Steering Committee. If there are extenuating circumstances that delay the start of work, the lead author should communicate this to the Steering Committee. Extensions can be granted if needed but should be accompanied by a clear timeline.

Once the proposal for a Working Group Paper is approved, the Lead Authors should:

1. *Recruit co-authors as planned.* Any invited co-authors should signal their intention to opt-in by responding by email to the lead author before the stated deadline. Lead authors are responsible for communicating regularly with co-authors about progress, including sharing drafts of analyses, figures, and text as often as is productive and practical.
2. *Create and use a GitHub repository for the paper in the HerbVar GitHub organization.* This will allow the authors to use HerbVar tools for accessing data, for standardizing analyses and manuscript preparation, and for keeping track of co-authors. We ask that authors use our GitHub organization to store and share code and data within their team and with the HerbVar Steering Committee. This helps ensure reproducibility and provides a secure backup for all data, work, and products.

Lead authors and their working groups should submit their paper to a journal within two years. If there are extenuating circumstances that delay paper submission, the lead author should communicate this to the Steering Committee. Extensions can be granted if needed but should be accompanied by a clear timeline.

Lead authors should circulate complete drafts among co-authors and consider comments and changes. Co-authors on papers with large numbers of authors should recognize the final decisions belong to the lead author, given that suggestions from co-authors may conflict.

Final manuscripts should be reviewed and approved by each co-author before submission.

All authors and co-authors should fill out their contribution in the authorship rubric and attach it as supplementary material to any HerbVar manuscript. Lead authors are responsible for ensuring consistency in credit given for contributions, and may alter co-author’s entries in the table to do so. An easy way to manage the author table is with an online spreadsheet. Note that the last author position may be appropriate to assign in some cases. For example, this would be appropriate for advisors of lead authors who are graduate students or postdocs and for papers that two people worked very closely to produce.

The lead author should carefully review the authorship contribution table to ensure that all authors have contributed at a level that warrants authorship and that contributions are consistently attributed among authors. Has each author made contributions in at least two areas in the authorship rubric? Did each author provide thoughtful, detailed feedback on the manuscript? Authors are encouraged to contact the HerbVar Steering Committee about any confusion or conflicts.

We encourage lead authors of working group papers to include as many co-authors as they can productively work with, to prioritize inviting HerbVar network members, and to

8. Proposing ‘Working Group’ Papers

prioritize inviting early-career scientists and scientists from under-represented groups or regions.

8.3. Identifying & inviting potential co-authors of Working Group Papers

HerbVar provides a tool to help lead authors identify potential co-authors to invite to assist with working group papers. All Site PI's are asked if they would like to be included in a database of individuals interested in co-authorship on small-group papers. The database lists name, career stage, institution and location, areas of expertise and/or topics of particular interest, number of HerbVar papers or manuscripts this person is an author on, what HerbVar datasets they have contributed to, and a link to their website or google scholar page or researchgate page.

Note that for any of the approaches listed below, authorship requires at least two types of contribution to a paper; see Qualifying for Authorship. **The approaches for inviting co-authors listed below are on a continuum from least to most inclusive.**

1. **Traditional.** The lead author invites known individuals with value for the particular paper. These individuals may not necessarily be HerbVar members.
2. **Traditional Plus.** The lead author invites known individuals with value for the particular paper, plus a few people chosen from the HerbVar database as a good match for this project.
3. **Application-based.** The lead author invites all HerbVar site PI's who have contributed data to be used in the project to apply to be one of some limited number of authors, and chooses applicants to create the best team.
4. **Opt-in: Limited.** The lead author decides on a number of co-authors they feel they can manage, and offers their paper to all HerbVar site PI's who have contributed data to be used in this project on an “opt-in” basis. Site PI's can opt-in until the desired number of collaborators is reached (first-come first-served).
5. **Opt-in: Open.** The lead author offers their paper to all HerbVar site PI's who have contributed data to this project on an opt-in basis. Any site PI who wants to participate is invited. If considering this approach, we suggest reading this paper about writing a massively multi-authored paper.

8.4. Instructions for the *Opt-in* approaches

The following steps should be used to identify and invite authors for the Opt-in approaches.

1. The lead author uses the HerbVar co-author database to identifies site PIs who have contributed data to be used in this project.

8. Proposing ‘Working Group’ Papers

2. The lead author circulates the manuscript proposal to the appropriate site PIs by attaching it as an email to the HerbVar email list. The subject line of the email should include the phrase “OPT-IN PAPER.” This email should also include a deadline by which time co-authors should respond.

Once individuals wishing to opt-in respond to the lead author, the lead author is responsible for working with all co-authors to organize the process and timeline for completing the paper.

8.5. When to invite co-authors

The right time to share your working draft and solicit co-authors is different for each manuscript, but in general:

- Sharing early drafts or figures allows for more effective co-author contribution. This would mean inviting co-authors at a very early stage.
- Circulating almost-complete manuscripts does not allow the opportunity for meaningful contribution from co-authors, and is discouraged.

8.6. Who should accept invitations to be a co-author?

Authorship must be earned through a substantial contribution. For co-authorship, each individual must contribute in *at least two ways* listed in the rubric below, including contribution to the writing process. Traditionally, project initiation and framing, data analysis and interpretation, and manuscript preparation are all authorship-worthy contributions, and remain so for HerbVar manuscripts. However, HerbVar collaborators have also agreed that contribution of data being used in a paper can be one factor qualifying a site PI for co-authorship, as long as the collaborator makes additional contributions to the particular manuscript, including data analysis, writing, or editing. HerbVar manuscripts will be accompanied by a supplemental table indicating authorship contributions. These guidelines apply equally to manuscripts led by graduate students.

Rubric item	Example contributions counting towards authorship
Developed and framed research question(s)	Originated idea for current analysis of HerbVar data; contributed significantly to framing the ideas in this analysis at early stage of manuscript
Analyzed data	Generated models (conceptual, statistical and/or mathematical), figures, tables, maps, etc.; generated a Network-scale dataset being used in this manuscript’s analysis
Contributed to data analyses	Provided comments, suggestions, and code for data analysis

8. Proposing ‘Working Group’ Papers

Rubric item	Example contributions counting towards authorship
Wrote the paper	Wrote the majority of at least one of the sections of the paper
Contributed to paper writing	Provided new text, help with organization framing, and provided citations linking to new literature areas
Site PI	Coordinated data collection, proofing, and submission of data for at least one site used in this manuscript
Network level coordinators	Contributed substantially to network level activities such as management of network data, recruiting and assisting new sites, finding funding for network level management activities

8.7. Authorship Disputes

The steering committee works to ensure communication across projects to avoid overlap of manuscripts, and provides guidance on procedures and guidelines. Disputes over authorship and manuscripts should be brought to the Steering Committee (Chapter 2) for resolution.

8.8. Manuscript Preparation

Please refer to Chapter 10 for details on the HerbVar Research Workflow and guide to preparing reproducible manuscripts. Chapter 12 includes required information to include manuscripts, such as citations of Herbvar datasets and acknowledgement of NSF support.

9. Proposing New Data Collection

HerbVar network members are welcome to propose new add-on data collection efforts by sending a proposal to the Steering Committee. The Steering Committee will review proposals to ensure the network is focusing its efforts on the most important data collection objectives with the highest probability of success.

9.1. Species & Site Priorities

Based on Phase 1 of data collection, we ask that you prioritize the following three objectives when proposing species for Phase 2 of data collection:

9.1.1. Objective 1: Focal species across gradients

Goal: Sample our three focal species – *Taraxacum officinale*, *Plantago major*, and *Plantago lanceolata* – across broad geographic and/or environmental gradients.

Justification: Sampling within these three species will increase the depth of our understanding of the effects of particular abiotic gradients (e.g., elevation, precipitation, temperature, etc.) as drivers of variability in herbivory pressure within plant species. Each species is standardized within itself so studies of herbivory across a range of contexts allows for strong inference on the effects of those contexts.

9.1.2. Objective 2: Novel species & contexts within focal families

Goal: Sample species from novel clades or with atypical growth forms within our five focal families – Apocynaceae, Asteraceae, Fabaceae, Rubiaceae, and Solanaceae. Surveys of plant species that were included in Phase 1 are also welcomed but should prioritize novel regions, habitats, or environmental contexts.

Justification: Increased resolution within our five focal families allows for testing of drivers of herbivory variability in a phylogenetically-explicit framework. Understanding the impact of evolutionary differences within these families on the distribution of damage will be crucial in teasing apart macroevolutionary patterns.

9.1.3. Objective 3: Damage to Reproductive Tissues

Goal: Survey damage to the fruits, flowers, and seeds of any species.

Justification: Damage to reproductive structures has—arguably—the most direct impact on fitness so an understanding of the drivers of variation in herbivory damage to reproductive tissues is crucial for understanding population-level or fecundity-related consequences of herbivory variation.

9.2. Responsibilities of Add-on Project Leaders

Leaders of add-on projects must commit to:

- Creating the data-collection protocol with input from the Steering Committee.
- Recruiting site PIs to collect sufficient data for at least one publication.
- Leading the writing of at least one paper using their add-on project data.
- Including all site PIs who contribute substantially to the project as co-authors on at least one paper using project data. In most cases, a substantial contribution would be at least three surveys, although if other data collection is required (e.g., plant traits, herbivore counts, etc.), then “substantial” will be defined by the project leaders. All individuals who contribute data must have their efforts recognized with authorship, assuming that they also contribute to one additional aspect of authorship (see Authorship guidelines).

9.3. Proposal and Approval of New Add-on Projects

The steps to proposing a new add-on project are as follows:

1. Review the current add-on projects and proposals for add-on projects to determine whether your idea is already being addressed. If not, proceed to step 2.
2. Email a proposal to a Steering Committee member (contact information in Chapter 2). The proposal should have:
 - i. Working title
 - ii. Current authors: including lead, co-leads, and additional authors.
 - iii. Abstract (~200 words) describing the knowledge gap, why it is important, and briefly describing the data to be collected to fill that knowledge gap and the protocol for collecting those data
 - iv. Data: What data from the HV database will be used (e.g., Phase I, Phase II, all, etc.) - Response variables - Predictor variables
 - v. Authorship model: We strongly recommend following the HerbVar core data authorship model, although many working groups may follow a different author model. Please see the authorship process here.
 - vi. Timeline - Data processing - Analyses - Writing - Submission

9. Proposing New Data Collection

The Steering Committee will review the proposal, ensuring there is no overlap with an ongoing data collection effort, and provide feedback. You will work with the Steering Committee to incorporate their feedback. If the proposal is approved, move to the next step.

3. Post your proposal for network members to read and email the network with “HerbVar add-on data project” in the subject line. Use some mechanism (return email, google form, etc.) to find out how many people want to opt-in to this project by contributing at least three surveys.
4. If you have enough people opting-in to make the project viable, inform the Steering Committee and the project will be moved to “in-progress” on the HerbVar website. Depending on the complexity of your data, the Steering Committee or you will update our data structures to receive the new data.
5. Create and post your protocol, with Steering Committee input.
6. Send another email to the people opting in to your project telling them to start data collection.

9.4. Evaluating Progress & Participation

The HerbVar Steering Committee will review ongoing HerbVar projects, working groups and site PI's periodically. Site PI's whose behavior does not align with HerbVar guidelines (e.g., not reporting data errors, not treating collaborators with respect) will be asked to leave the project. Working Group leaders will be responsible for sharing their progress (e.g., manuscript drafts, results, data, code) with the Steering Committee when requested. If working groups do not make progress on their project within the first 6 months after a manuscript is proposed, or if they do not submit a manuscript within 2 years after proposal, the Steering Committee may suggest that the working group change its approach, or open the project to others who are interested in carrying it forward.

Part III.

HerbVar Workflow

10. Research Workflow

HerbVar relies heavily on version control with Git/Github to facilitate data validation and management (Goodman et al. 2014, Yenni et al. 2019, Kim et al. 2022), promote collaboration (Boland et al. 2017, Braga et al. 2023), and streamline data access, analysis, and archiving (Champieux et al. 2023). Below we describe the workflow for (a) collecting data for HerbVar and uploading it for integration with the global data set and (b) analyzing some or all of the global HerbVar data set and preparing a manuscript based on these analyses.

10.1. Workflow: New analysis with Herbvar Datasets

1. Identify Questions & Submit for Review (see Section 9.4) to ensure no overlap with ongoing projects.
2. Create a repository for harvesting, organizing, and analyzing the data to be used in the analyses using the [new_analysis_and_paper_template](#) (see Section 11.1).
3. Write the manuscript following using the resources and guidelines in Chapter 12.

10.2. Workflow: Collecting new HerbVar Data

1. Review and Select Protocols (see Chapter 13).
2. Collect Data.
3. Create a repository for cleaning and analyzing the data using HerbVar's [new_dataset_template](#) (see Section 11.1).
4. Validate the data using the procedures in Chapter 11
5. Upload the clean version of the data using the [Herbvar Data Portal](#)(see Section 11.2).
6. Write the manuscript following using the resources and guidelines in Chapter 12.

11. Data Validation & Upload

11.1. Data Validation & Analysis

Instructions for using setting up a github repository for data analysis and making reproducible corrections using scripts.

11.2. Uploading Data

To upload data, please visit our [data submission portal](#) and be sure to use the template Excel file linked in Chapter [13](#)

12. Preparing Manuscripts & Presentations

Write manuscripts following the HerbVar guidelines for authorship (Chapter 7) and use the checklist below to ensure all required information is included. Note that the *HerbVar Data Analysis Template* includes the files and code needed to write the manuscript in using Rmarkdown templates for scientific papers (e.g., `papaja` or one of the `rticles` templates).

12.1. Checklist: Prior to Submission (Manuscripts & Presentations)

- Author Lists: Verify the Name, Institution, and OrcidID of all authors. If full lists of authors are not feasible (e.g., on oral presentation abstracts) or allowed by the journal, a short authorship list can be published with “The Members of the Herbivory Variability Network” as a coauthor, and a link to the full HerbVar author list. If possible, include the full author list in the supplement.
- All HerbVar publications must include the following text in the Acknowledgements:

“This work was generated using data from the HerbVar Network (<https://herbvar.org/>), which was supported by the National Science Foundation (Grant No. DEB-2203582).
- If the data set used in the manuscript has been archived in a repository, please cite both the data set archive and the original article in your paper section. For example, if using the Core Dataset archived at Dryad, your manuscript or presentation should include citations to both of the following:

Wetzel, William; Hahn, Philip; Inouye, Brian et al. (2023). Plant size, latitude, and phylogeny explain within-population variability in herbivory [Dataset]. Dryad. <https://doi.org/10.5061/dryad.44j0zpckm>

The Herbivory Variability Network (2023). Plant size, latitude, and phylogeny explain within-population variability in herbivory. *Science* 382, 679-683. DOI:10.1126/science.adh8830
- Please cite the HerbVar Manual in the Methods section when describing how data were collected in the field.

HerbVar Steering Committee (2024). “HerbVar: Project Manual and Field protocols (v0.9.0)”. Zenodo. <https://doi.org/10.5281/zenodo.14232308>

12. Preparing Manuscripts & Presentations

- Keywords: **Please use “HerbVar” as one of the keywords when submitting manuscripts.** This allows products using HerbVar to be more easily found when the articles are indexed in databases such as SCOPUS.
- Add the MS or Presentation to the Herbvar Zotero Library (Important for NSF reporting to know how many manuscripts are in review and presentations have been given.)
- Post a Preprint or the Slides (optional but encouraged)

12.2. Checklist: Upon Acceptance (Manuscripts)

- Update the record in the Zotero library
- Archive any ‘new’ Data with Dryad
- Include a citation of the ‘new’ data’s Dryad archive in the accepted version of the manuscript in accordance with the journal’s guidelines (e.g., in the *References*, *Data Availability Statement*, etc.).
- Archive the Analysis Repo on Zenodo. instructions on freezing the data analysis code repo on Zenodo can be found [here](#)
- Include a citation of the Analysis Repo in the accepted version of the manuscript in accordance with the journal’s guidelines (e.g., in References, Data Availability Statement, etc.).
- Archive the Manuscript Repo on Zenodo (optional). instructions on freezing the MS repo on Zenodo can be found [here](#)

Part IV.

HerbVar Protocols

13. Protocols Overview

! Important

Below are links to the HerbVar sampling protocols. Please be sure to review the larger strategic vision and project goals of the HerbVar Network, which are summarized in Chapter 1.

13.1. Species & Site Selection

Our approach to select plant species and/or sites to survey is described in Section 9.1. Briefly, we suggest collaborators strive to survey one of the following:

1. One of our three focal species – *Taraxacum officinale*, *Plantago lanceolata*, and *Plantago major* – in a novel geographic/environmental context,
2. New clades or growth forms of species in our five focal families: Apocynaceae, Asteraceae, Fabaceae, Rubiaceae, and Solanaceae, or
3. Reproductive tissue damage of any species.

13.2. Primary Protocol

The **Primary Protocol** for collecting HerbVar data with most species (e.g., sample sizes, selecting individuals to sample) is described in Chapter 14. If there is a reason why you think this protocol will not work for your species or in your field sites, please consider one of the alternative protocols below or contact the HerbVar Planning Group.

Estimating damage by herbivores: Chapter 15 provides a detailed walk-through of the process for estimating herbivore damage on leaves and whole plants, including suggestions for different types of leaves and damage. There is also an *Illustrated Guide to Percent Leaf Damage* (download link: Section 23.1) to help estimate different levels of damage to leaves. *We suggest printing this guide and taking it with you to the field to help estimate percent herbivory.* It currently includes leaves of two species as examples; it will be updated with examples from other species.

13.2.1. Datasheets

1. **Datasheet - Excel File** (download link: Section 23.1). This Excel file is a template datasheet designed to work for the HerbVar Primary Protocol. It contains a “data dictionary” sheet that defines all columns if any abbreviations are unclear.

13. Protocols Overview

2. **Datasheet - Printable PDF** (download link: Section 23.1). We have split the printable datasheets into three parts, one each for the Primary, Reproductive, and Herbivore Protocols. The herbivore datasheet is built for you to print as many copies of the second page as you have identified herbivore groups.

13.3. Damage to reproductive tissues

If your plants have reproductive tissues (e.g., flowers, fruits, seeds), please follow the protocol in Chapter 16 to quantify damage to these tissues.

13.4. Alternative survey protocols

13.4.1. Rare Plants

Chapter 17 describes protocols for rare plants (i.e., plants found at low densities / abundances).

13.4.2. Cacti & Other Succulents

Chapter 18 describes protocols and issues related to quantifying herbivory on cacti and other succulents.

13.4.3. Trees

Chapter 19 is a protocol for surveying mature trees. It also discusses how to handle seedlings and saplings of tree species. If you are sampling tree species in their seedling or sapling stage (i.e., <2m tall) please refer to the Primary Protocol.

13.4.4. Rhizomatous species

Chapter 20 is a protocol for rhizomatous species for which it is feasible to determine what constitutes a genet (e.g., by identifying rhizomatous connections) and for which genets are small enough that herbivory could be estimated on ~30 genets in a site and their nearest neighbors.

13.5. Sampling Insects

All surveys should note internally-feeding herbivores (e.g., gallers and miners), but only some should take the extra time to sample external herbivores. Chapter 21 discusses *whether and how* to sample insects, and includes visual cheatsheets for several identifying several focal groups of insects and for counting galls and mines.

13.6. Entering and Correcting Data

Do not make any changes to the raw data files once you have entered the data.
Make any corrections using R scripts. For additional information on how to do this, see Chapter 10

14. Primary Protocol

14.1. Overview

Below, we provide a straight-forward and broadly applicable protocol to achieve project goal of quantifying variability in plant-herbivore interactions. This is HerbVar's Primary Survey Protocol. In brief, 30 randomly selected plant individuals in a site (~population) are surveyed for herbivore damage and (possibly) herbivore abundance. Data are also collected on the nearest conspecific neighbor of each plant (for a total of $N = 60$ plants). These methods yield estimates of variability, skew, and spatial patterns (e.g., autocorrelation) in herbivore damage. If the Primary Protocol is not feasible for a species or site, then we suggest one of the alternative protocols.

! Alternatives to the Primary Protocol

The HerbVar Primary Survey Protocol is designed to work for many common plant growth forms and contexts, so we expect most surveys to use this protocol. The primary protocol, however, will not work for every plant growth form or context, so HerbVar has multiple alternative survey protocols. These include protocols for surveying the following:

- Reproductive damage: Chapter 16
- Low density/abundance populations: Chapter 17
- Cacti and other succulents: Chapter 18
- Mature trees: Chapter 19 (*surveys of immature trees (i.e., seedling/saplings) use the Primary Protocol below*)
- Rhizomatous geophytes: Chapter 20
- Insect herbivores, galls, and mines: Chapter 21

If none of these alternative protocols fits the situation, then collaborators may deviate from the primary protocol. We trust collaborators to decide how to adapt the primary protocol in ways that work for their systems. We suggest, however, that collaborators strive to follow the spirit of the protocol below: randomly select at least 30 plants from a site and census them and their nearest neighbors for herbivory and herbivore data. For a dataset to be usable in the overall study, it will have to be comparable to data collected using this protocol. Collaborators who deviate from the HerbVar protocols should carefully record their methods.

14.2. Preparing to Sample

14.2.1. Select a plant species

We have developed the following objectives based on the patterns found in the first phase of data collection:

1. Surveys of the three focal species (*Taraxacum officinale*, *Plantago lanceolata*, and *Plantago major*), especially across broad environmental and/or geographic ranges
2. Surveys of species in the five focal families (Apocynaceae, Asteraceae, Fabaceae, Rubiaceae, and Solanaceae). We want surveys of new species within these families, especially species from new clades or with unusual growth forms. For repeat surveys of species within these families, we are prioritizing surveys from new regions, habitats, elevations, etc.
3. Surveys of damage to any species' reproductive tissues (e.g., flowers, fruits, etc.).

While we welcome all surveys, data that fall under one or more of these three guidelines is particularly valuable in addressing the current scope of HerbVar's research questions. Please refer to our more detailed HerbVar Species Selection Protocol for more information on species selection and how data contribution relates to authorship in papers utilizing those data.

14.2.1.1. A note on the ideal abundance of focal plants

The primary protocol works best for sites with at least ~90 plant individuals, which allows for random sampling. If your site has fewer than ~90 individuals of your plant species, then please consider conducting a comprehensive census of all individuals within the site as suggested in the Low Density/Abundance Protocol. A comprehensive census, when feasible, would be even better than the protocol below. If plants are far enough apart, please take GPS coordinates for each plant. If a comprehensive census is not feasible, then please modify the primary protocol or the low-density/low-abundance guidelines to work efficiently with your species and site. Please reach out to the HerbVar coordinators if you have questions or want to check that your modifications will lead to adequate data.

14.2.2. Learn to Estimate Herbivory

First, review HerbVar's Damage Estimation Training Guidelines (see Chapter 15), which contains valuable information on how to estimate percent damage on various leaves, the precision of estimates, and acceptable binning. **Next, train and test team members with the ZAX Herbivory Trainer.** This web-based application, created by Dr. Angela Moles and Zoe Xirocostas, provide a risk-free environment for testing oneself on per-leaf damage estimation. Note that the app prompts you to assess damage to the nearest percent while our protocol is slightly coarser (see Table 15.1 of the Damage Estimation Guidelines). The app has two stages - in the first you assess damage on a leaf and are immediately told how close you were to the correct amount, while in the second you are given the results

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after estimating herbivory on 50 leaves. Please feel free to focus on the first part of the app until you are confident (though you are of course welcome to do the second if you want extra training). Finally, **download and familiarize yourself with the datasheets for this protocol.** There are digital (see siteData, densityData, and plantData sheets) and printable versions to facilitate standardized data entry. If you have a question, feel free to reach out to herbvar@gmail.com.

14.2.3. Site Selection & Delineation

A “site” should be an area where a given plant species occurs at a high enough density to easily select 30 focal plants and 30 unique neighbors with our method. While new sites can be anywhere in the world, the most valuable new sites are those representing geographic regions, environmental conditions, habitats, or other ecological characteristics absent from or poorly represented in our database. This is especially important for our focal species and re-surveys of other species we already have in our database. It’s less important for new species, especially when those new species are from clades that we do not currently have in the database.

We recognize that defining the ‘edges’ of a site can be subjective and not easy. Typically, we identify an area where the focal plant species occurs at a sufficiently high density to allow for selecting 30 focal plants and 30 unique neighbors using our search methods. This is usually a relatively dense patch. Walk around and see if you see the density drop off to well below the mean density that is used to calculate radius size. This is usually quite simple, e.g., when we walk out from the center of a “site” and don’t see any individuals of the focal species within 5 m, we decide we’re at the edge of a patch. In some systems, delineating a single, sampleable population simply might not be possible (e.g., where a species covers a vast area). In these cases, collaborators should simply do their best to select a reasonable, representative area to sample.

14.2.4. Pick a time to sample

The optimal time to sample will depend on the natural history of the system. Data can be collected at any time as long as there has been some herbivory. We will use the sampling date to examine how herbivory changes seasonally (please note approximate dates for beginning and end of growing season for each survey, see siteData sheet in datasheet template). However, the most valuable surveys will be after enough time has passed for an ecologically meaningful amount of herbivory to accumulate. In strongly seasonal systems, this will be in the latter half of the growing season. But it could also be once leaves have reached maturity (e.g., for species in which most herbivory is on expanding leaves). In other systems, the best time to sample might be during or after a key life history stage (e.g., flowering). All that said, there is no perfect time to sample. Collaborators should use their knowledge to decide when to sample. Repeat sampling is acceptable.

14.2.5. Determine a radius for your circular quadrats

If you are sampling one of the HerbVar focal species (*Taraxacum officinale*, *Plantago major*, *Plantago lanceolata*): Use a radius of 0.4 m for your quadrats. This will standardize across surveys of these same species. Note that if your populations are sparse, you may use a larger radius following the other process or pre-calculated values (Table 14.1).

If you are using any other species, use the following process to determine a quadrat radius or use the table below:

1. Select 10 random locations in the site.
2. At each of these points establish a 1 m² quadrat and count the number of plants in each quadrat
3. Calculate the mean density of plants in the quadrats, D ,
4. Calculate a circular quadrat radius (r) that would – on average – contain 4 plants using this formula: $r = \sqrt{4/D}$.

This approach seeks an optimal, intermediate quadrat size that balances the costs associated with a small quadrat size (many empty quadrats) and a large quadrat size (quadrats that require counting many plant individuals).

Instead of calculating as above, you may also use this pre-calculated set of radii (Table 14.1) for non-focal species. **Remember, for focal species, please use 0.4 m**

Table 14.1.: Table 1. Pre-Calculated Quadrat Radii

Plant Density (D , plants / m ²)	Quadrat radius (r , meters)
$D \leq 0.1$	3.6 m
$0.1 < D \leq 0.25$	2.9 m
$0.25 < D \leq 0.5$	1.9 m
$0.5 < D \leq 1$	1.35 m
$1 < D \leq 3$	0.9 m
$3 < D \leq 6$	0.55 m
$6 < D \leq 10$	0.4 m
$10 < D \leq 20$	0.3 m
$20 < D$	0.23 m

14.3. Sampling

1. Lay a transect through the middle of the site (Figure 14.1). Record (i) the GPS coordinates of the transect origin, and (ii) the length (m), and compass direction (degrees) of transect (need to pick a coordinate system and precision)
2. Select center points of circular quadrats (Figure 14.1). Randomly select 40+ points in the site by selecting pairs of random numbers. One random number represents distance along the transect (0=length of transect); the other represents distance left or right of the transect (left=negative, 0=center, right=positive). These are the center points of quadrats.

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3. Locate the center point of each quadrat, then count and record the following quadrat-level data:
 - a. **The number of focal plants within r meters of the center point** (see Figure 14.1). See above for explanation of how to calculate r , or use the values in Table 14.1. It may be helpful to place a stick vertically in the center of the quadrat, attach a string of r meters to the tip, and walk in a circle around the stick to help visualize the circular quadrat. **Note:** this includes only rooted focal plant species individuals in the quadrat.
 - b. **The percent cover of focal plant species** (ignore non-focal species). **Note:** this includes both rooted and not rooted focal plant species individuals in the quadrat but hanging over the edge from above. This percentage could be $> 100\%$ if plants overlap.
 - c. **The percent cover of all non-focal plant species** (ignore focal species). This percentage could be $> 100\%$ if plants overlap. If surveying understory plants, ignore forest canopy when estimating percent cover.
4. If the quadrat has > 0 of the focal plant species: Randomly choose 1 of the plants in the quadrat to survey (a faster alternative would be to choose the plant closest to the quadrat center, but this is recommended only if you think it will produce an unbiased sample of plants from your site. If you chose this approach, be careful about over-representing isolated plants or larger ones, which in crowded patches will be closer to more points relative to small plants). **For the selected plant record:**
 - a. **The plant's life-history stage** (i.e., seedling, vegetative, flowering, fruiting). Note that if multiple stages are present, record all relevant stages (i.e., a plant can be both flowering and fruiting).
 - b. **The size of the plant.** Use judgment to pick best measure for your species (e.g., plant height from the ground to tallest living part, stem length, foliage diameter, stem diameter).
 - c. **The following three measures of herbivore damage** (see also Chapter 15). Note that “herbivore damage” includes damage caused by both vertebrate and invertebrate herbivores.
 - i. **Presence/absence of leaf damage:** Record both the total number of leaves on the plant and the number of leaves with any visible damage ($> 0\%$ herbivory). **Note that we are no longer including undamaged leaves in step ii below, so the presence/absence data are vital in understanding the proportion of the plant that is damaged by herbivores.** If the plant has ≤ 60 leaves total, please count and score all leaves in this step. If a plant has > 60 leaves, then scoring all leaves may be too time-consuming. In that case, you can subsample leaves to score for this step. If you choose to subsample leaves for this step, then randomly select 60 leaves and score them for presence/absence of damage. Please also select “subsample” for the “subsample_or_allLeaves” column (see template datasheet). If you count and score all leaves, select “true total” for that column.

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ii. **Estimated percent damage on 10 randomly chosen leaves with herbivore damage** ($> 0\%$ herbivory). One estimate per leaf (for a total of 10 estimates). Strive to ensure the selected leaves are representative of all leaves on the plant (e.g., sample young and old leaves in proportion to frequency on plant). If desired, you may use an application to estimate damage (e.g., Leaf-Byte, etc.). However, please make a note of that in the appropriate part of the siteData tab of the template datasheet. **Note that all selected leaves should have $> 0\%$ damage** (this is a change from the Phase 1 protocol). Note also that measuring only damaged leaves makes the data collected in step 1 (see above) vital in understanding per-plant damage variation.

If damage is estimated visually, and leaves are visibly damaged but damage is $\leq 0.5\%$, record herbivory as 0.5%. If a leaf has $< 0.1\%$ damage, round that down to zero. If using an app, make a note and put in the value the app provides. For leaves with herbivore-built leaf shelters (rolls and ties), please carefully peer into or open shelters to estimate damaged area and count resident herbivores.

- iii. **Estimated percent damage across the whole plant.** For example, if a plant has 4 equally sized leaves of which 2 are 50% eaten, then herbivory for the whole plant is 25% (if leaves are different sizes, correct this estimate by taking leaf size into account). If this measure is not feasible to collect, measure 30 leaves (instead of 10 as in step 2 above) and leave this blank. The 30 can then be used to calculate this value *post hoc*. **Optional but valuable:** separating percent damage by herbivore type or species, if possible (e.g., sucking damage versus chewing damage); add columns as needed.
- d. **Optional but encouraged:** Estimate and report damage to reproductive parts (flowers/fruits/seeds) using the Protocol in Chapter 16.
- e. **Record the presence of plant diseases (i.e., pathogens).** Please also estimate your confidence in your pathogen estimate and include it as a note in the provided column in the datasheet. In Phase 1, several collaborators pointed out that the difference between pathogen pressure and nutrient deficiency can be slim so this confidence estimate will be helpful in accounting for the difficulty in pathogen estimation.
- f. **Record number of leaf mines and galls per plant.** If there is reason to believe that galls or mines have accumulated through multiple years (e.g., stem galls on woody perennials), please note this. If there are too many mines or galls to count individually, estimate the number per plant by tallying the number per module (e.g., stem, branch) and multiplying by number of modules. If serpentine/linear mines cannot be confidently recorded, instead count only blotch mines to record a consistent mine abundance (see visual guide at bottom of Herbivore Sampling Protocol for definitions of “serpentine” versus “blotch” mines). **Optional:** abundance of other externally-feeding herbivores (standardized approach; see Herbivore Sampling Protocol to decide if/how to collect these data).
- g. **Record the distance to nearest conspecific neighbor** (where the nearest neighbor is the plant with the closest above ground tissue to any aboveground tissue on the focal plant).

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5. Identify the nearest conspecific neighbor of the selected plant and record the same data as for the focal plant except for the data on “neighbor” plants (i.e., record nothing about the “neighbor’s neighbor”).
6. Continue visiting the randomly selected points until ≥ 30 focal plants and 30 nearest neighbors have been surveyed.

14.4. After the Field

1. Enter your field-collected data into the “Template Excel file” (link at Section [23.1](#)). Refer to the data Dictionary sheet if column meanings are unclear.
2. Use the Data Submission Portal to upload your data. The portal has numbered steps to assist the upload process (for instructions see Chapter [10](#)).
3. After uploading via the submission portal, check the Completed Surveys file to ensure that your data were uploaded successfully. Uploaded data will have your entries in the sidebar of the app as the bottom-most row of that file.

14.5. Methods Notes

1. Modifications of this protocol may be necessary to adapt it to different systems (see “Alternate Protocols” box above). If the primary protocol won’t work for your system, please first consult our alternative protocols. If our alternative protocols do not solve the issues, then you may adapt the primary protocol as needed. **Whatever you do, please record methods carefully and strive to follow the spirit of the protocol and produce comparable data.**
2. Collaborators have reported that one survey (~60 plants) takes between 0.5 and 2 person-days (i.e., 4-16 hours) using the methods above (after a species and site have already been selected).
3. We select 40 quadrat center points (instead of 30) so that we have extra points ready in case some quadrats are empty. If you predict that many quadrats will be empty (e.g., in a very spatially clumped population of plants), then select more points (e.g., 60 points). (Remember the goal is to have 30 focal plants sampled, plus their nearest neighbors).
4. Sometimes, especially in small populations, a focal plant ends up being another focal plant’s neighbor. This is fine. Just note and keep going. If you have time, you can add an extra focal plant at the end (but this isn’t totally necessary).
5. For clonal plants, we have been calling stems “individuals” if they are not connected aboveground. When looking for above ground connections, we clear away detritus, but we do not dig or move soil. There is also an alternative protocol for surveying such species (see Chapter [20](#))
6. Please see our Damage Estimation Training Guidelines for guidelines on how to estimate herbivore damage. Here are two tips:

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Figure 14.1.: A diagram of the sampling scheme. (1) Record plant density in 10 randomly located $1m^2$ areas to estimate average plant density D , which is used to calculate quadrat radius r . (2) A quadrat with one focal plant and its nearest neighbor (outside quadrat). (3) A quadrat with no focal plants. (4) A quadrat with 5 focal plants; plant 3 is randomly selected for data collection, and its nearest neighbor is plant 4. Diagram by Moria Robinson.

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- a. Sometimes discerning herbivore damage from physical damage (e.g., wind, trampling) is tricky. We do the best we can. We look at things like how jagged the cut edges are and if they travel past the missing area into the remaining leaf tissue (which would suggest the damage may have been physical).
 - b. Another challenge is old damage that occurred when leaves were still expanding. This could potentially make the area removed seem larger than it was. If we suspect something like this happened, then we try to bend the leaf back into shape to see if it seems like the missing area expanded over time.
7. We will accept surveys that only assess damage and do not identify herbivores. This will allow people without insect ID skills to participate in the study.

14.6. Common Garden Data

Common gardens are a powerful tool for studying plant–herbivore interactions. Several collaborators have proposed including them in HerbVar, and we would like to try if we can get enough data. To be applicable to this study a common garden’s design would have to be random with respect to genotype. If a garden was somehow stratified with blocks containing repeated instances of, e.g., different levels of leaf toughness, then damage distributions will not be comparable to damage from wild populations. We may still be able to use such datasets, but only if we have enough to use them in a separate analysis. Please get in touch if you would like to contribute common garden data.

15. Estimating Damage

15.1. Overview

So, you've figured out how to find the random set of plants to score damage on (e.g., using the Primary Protocol). Now it is time to look closely at each plant in order to score the amount of damage caused by herbivores. This is a task that will vary among plant species. This document is meant to guide you through the process of estimating herbivore damage using best practices that we have developed. Don't fret that this document looks too long to follow for 60 plants per survey: we give an overabundance of pointers to cover likely hiccups that could occur across the world's 400,000 plant species. Estimating herbivory will become second nature after you've familiarized yourself with the methods and had some practice. Ideally with these tips you should be estimating damage on a single plant in less than five minutes (and maybe a lot less!).

In this document, we provide detail for four key steps from the Primary Protocol:

1. Estimating plant size (and determining what tissue counts).
2. Counting number of leaves and number of damaged leaves (up to 60 max).
3. Estimating percent damage on 10 randomly chosen leaves.
4. Estimating percent damage across the whole plant

Note

If you are just looking for quick tips on estimating percent damage, skip to “*Estimating Percent Damage on 10 Randomly Chosen Leaves*” below.

Two related documents:

1. The “*Field Guide to Different Types of Herbivore Damage*” (Chapter 21), which has photos of different types of damage
2. An “*Illustrated Guide to Percent Leaf Damage*”, which shows images of leaves with different percent damage to help you calibrate your visual estimates.

Objectives: First off, it is good to keep in mind the goals of these estimates: Ideally, we would like to know (1) the total amount of plant material consumed by herbivores (e.g., grams of tissue eaten) *and* the (2) total amount of plant material remaining (e.g., grams of tissue remaining). Unfortunately, this is not feasible for most systems, so our goal is to approximate them by estimating (1) plant size and (2) percent removed by herbivores for entire plants *and* for a sample of leaves *within* plants.

💡 What kinds of plants will this work for?

This document is written with a focus on vegetative tissue on leafy plants < 2 m in height. If your plants fit this, then great; read on!

If your species is a tree or large shrub, you have two options: First, we recommend most HerbVar collaborators restrict themselves to seedlings and saplings, which we define as < 2 m in height. In this case, just ignore any individuals > 2 m tall at your site, and follow the methods below. Make sure to note that you were excluding mature individuals. If you want to include mature trees (> 2 m) in your survey, then please follow the HerbVar Tree Protocol (Chapter 19). The Tree Protocol describes a method for estimating herbivory on a subsample of leaves on each tree. However, after you've chosen your subsample of leaves, you will still have to estimate damage on each leaf, so the damage estimation tips and illustrated guides below will still be very helpful.

If your species is a cactus or other succulent, then please see the Cactus and Succulent Protocol (Chapter 18).

If your plants have reproductive tissues (flowers, fruits, seeds) and have had them long enough to potentially sustain herbivore damage, then please see the HerbVar Reproductive Damage Protocol (Chapter 16) for how to record this damage. The rest of this document focuses on damage to vegetative tissue.

15.2. Estimating Plant Size (and Determining What Tissue Counts)

In many cases, it will be clear what an individual plant is, but in cases of clonal plants, we will consider each ramet (i.e., above-ground unit) to be a unique “individual.” Move leaf litter to look for above-ground connections, but do not clear away soil.

In most cases, herbivores do not eat all plant biomass. Therefore, it will be useful to note the tissue that you are measuring damage on. For most HerbVar surveys, this will be vegetative tissue: “leaves” and maybe “stems.” The key is to collect your damage estimates across all of the tissue within a plant or on a random sample of the tissue within a plant. Before you start estimating damage, give some thought as to precisely what to include. For example, it is probably best to avoid senesced leaves (Figure 15.1) unless perhaps the leaves were recently senesced such that they have not changed in size or distorted in any way. If you need to make any decisions about what to count, please remember to put detailed notes in the notes tab of the datasheet.

Once you have decided the extent of plant you will be surveying, you can measure plant size. Because there are so many different plant growth forms, we suggest using your judgement to pick the best measure of plant size for your species. Examples of measures that work well for many species are standing plant height (e.g., ground to tallest living part), stem length (better than standing height for creeping species), foliage diameter, and stem diameter. Just make sure to be consistent within a survey, and to detail your plant size measure in the notes.



Figure 15.1.: Senesced *Lepidium* leaves. Leaves like this are probably best excluded from damage estimates.

15.3. Counting Number of Total and Damaged Leaves (up to N = 60)

The first damage assessment step in the HerbVar Primary Survey Protocol is estimate the proportion of leaves with any damage, which we are defining as $> 0.5\%$ of a leaf removed by herbivores. We estimate the proportion of leaves damaged by counting the number of undamaged and damaged leaves on each plant (recording total number of leaves and number of undamaged leaves) up to a max of 60 leaves per plant. See the following sections and illustrated guides below for tips on how to decide if a leaf has more than or less than 0.5% damage. Here we'll discuss how to choose leaves to examine.

15.3.1. Plants with small number of large leaves (N = 1-3)

If you have a plant that has a small number of large leaves (e.g., 1-3), then the proportion of leaves with damage is not going to be a very meaningful estimate of overall herbivory. In this case, consider counting leaflets (instead of leaves), if your plant has leaflets. Otherwise, proceed with leaves.

15.3.2. Plants with <60 leaves

If your plant has < 60 leaves, we encourage you to quickly count and scan all of the leaves on the plant to look for the presence of herbivore damage. This will be easy on small plants and harder on large plants. Either way this step should take less than 2-3 mins. If it is too time-consuming to look at all the leaves on your plants or even up to 60 leaves (e.g., leaves are large or complex), then please pick a feasible number of leaves to subsample, ideally at least up to 30.

15.3.3. Plants with > 60 leaves

If you are restricted to examining a subsample of leaves within plants (because your plant have > 60 leaves or because it would be too time-consuming to do all leaves, then you'll have to decide how to subsamples leaves within plants. First, you will want to note the size of the subsample. Ideally you will have one number that will work for all plants in a survey. In that case, please detail this in the notes. If you need different subsample sizes for specific plants, please note subsample size in the notes for each plant, and please also make a note in the notes tab saying that you had to modify the number of leaves examined for some plants. Next you'll need a way to subsample leaves more or less randomly within plants. **If you need to subsample within plants, here are four potential methods:**

15.3.3.1. Subsampling Method 1: Nose-Pointing

Ian Pearse's nose-pointing method: For large plants, I like to choose four positions around the plant roughly at the cardinal directions (this never comes out as neatly as I might like since a lot of plants just don't grow that way). I stand at each of those positions, I turn away from the plant, I close my eyes, and I put my finger against my nose, like this (Figure 15.2). Then, I turn facing the plant, open one eye, and I choose whatever leaf I am pointing to (or the closest leaf if I'm pointing to multiple or none). I've done this on a lot for leaves, and I think it would basically work for other tissues (twigs, flowers, etc). You can continue to do this until you have examined your full subsample of leaves. Caveats: It is important to include mostly-eaten leaves using this method, but the method probably underestimates damage because you are less likely to be randomly pointing at a mostly-eaten leaf-nub.



Figure 15.2.: Nose pointing method to acquire a random sample of plant parts. (A) The researcher establishes a fixed point in his field of vision with an “L”-shaped hand position while turned away from the plant. (B) They then turn to the plant, open a single eye, and chooses the leaf that they are pointing to.

15.3.3.2. Subsampling Method 2: True Randomization

True randomization: Assign all leaves, seeds, etc. on the plant a random number, draw N numbers, and measure damage on those leaves. Caveats: this is rigorous, but probably too

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time consuming for most plants (and you'd probably just as well measure the damage on all the leaves you've given a number to!).

15.3.3.3. Subsampling Method 3: Arbitrary Sampling

Arbitrary sampling. That sneaker-word, arbitrary! This is basically to say "I really tried to choose an unbiased sample of the plant tissue, but I have no idea whether or not I succeeded." Caveats: Clearly, this can have problems, but it's what we're probably left with in most cases where plants have complicated architecture, the tissue is hard to choose in a more truly randomized way, or you're just strapped for time.

15.3.3.4. Subsampling Method 4: Define Your Own Method

Design (and make notes of!) your own subsampling scheme. Can you choose every seventh (or random-numbered) leaf along a shoot of skunkbush sumac (right)? Note how you did it, and approximately how much of the plant tissue you sampled (e.g., % of poison ivy sampled).



Figure 15.3.: Skunkbush sumac (*Rhus trilobata*)

💡 Philosophical thoughts about counting damaged leaves

This method works well (we think) as an estimate of overall herbivory for plants with small leaves or leaflets (e.g., sagebrush, *Astragalus*, locust), but will be very imprecise for plants with fewer, larger leaves because most large leaves will have some damage, though maybe not much. So, this is probably ineffective for your Welwitschia or banana tree. However, because counting a few large leaves is easy, we suggest doing it anyway for completeness.



Figure 15.4.: Locust (*Robinia*)

15.4. 3. Estimating Percent Damage on 10 Randomly Chosen Leaves

The next damage assessment step is estimating percent damage on 10 randomly chosen leaves. Well actually, the 10 data columns for this step (percLf1–percLf10) come after the column for whole plant percent damage (percHerbPlant) in the `HerbVar` template datasheet, but it makes more sense, in this document, to discuss percent herbivory on individual leaves before discussing whole plant percent herbivory.

1. If your plant has 10 or fewer leaves, then please examine them all.
2. if your plant has more than 10 leaves, then use one of the methods above to choose 10 leaves randomly. Strive to have these leaves be an unbiased subsample of all the leaves on the plant.

Finally, the main event—this is probably the main reason you’re reading this document. *There are many ways to estimate percent damage on leaves. For HerbVar, we recommend collaborators use visual estimation because other methods are slower and would make the sample sizes we need to describe herbivory distributions unattainable.* Moreover, careful visual estimation does a surprisingly good job, especially after some practice, especially if the primary goal is to compare the frequency of plants with low and high herbivory, as ours is. However, we strongly recommend checking your estimates against estimates from other observers using the same method, or even better against estimates using digital methods (i.e., LeafByte) to get a sense for how good of a job you are doing, and if you are overestimating or underestimating.

15.4.1. Visual estimation

Visual estimation is essentially as simple as it sounds. You look at a leaf and eyeball what percent was removed or damaged by herbivores. The benefit of this method is that it is quick,

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allowing us to obtain the large sample sizes we need to describe whole herbivory distributions. The caveat is that this method has more measurement error than other methods. For example, Zoe and Julie and Marc Johnson have found that visual estimation tends to overestimate damage by a few percent, particularly for researchers with less experience. We hope to mitigate estimation error with the guidelines below, our Illustrated guide to amounts of percent damage, an online training quiz, and a ground-truthing effort. **First**, we explain how to estimate damage. **Second**, we explain how to make sure you're doing a good job.

15.4.1.1. How to estimate damage.

1. Record estimates at a high resolution. We usually record at a resolution of 2.5% (Table 15.1). This may seem like unreasonably high resolution when you first try, but with a little practice and calibration you will get surprisingly good. We encourage trying for high resolution because—even with considerable error—high resolution estimates will likely be closer to the true values on average than estimates reported as broad categories (e.g., 0%, 1-10%, 11-25%, 26-50%... too coarse!). Plus, if you report your best guesses we can model the error statistically; we're out of luck if you just report broad categories.

Table 15.1.: Recommended resolution for recording percent herbivory

Herbivory (%)	Meaning
0	No herbivory
0.5	trace amount
1	~1 % herbivory
2.5	~2.5 % herbivory
5	~5 % herbivory
7.5	~7.5 % herbivory
...	...
100	everything removed except base of leaf petiole

2. When you first look at a leaf, do a quick mental calibration before estimating damage. We do this by visualizing cutting the leaf into a range of proportions. Start with large proportions and scale down to your finest resolution (e.g., 2.5%). For example, think about what half the leaf would look like, then imagine a quarter (25%) of the leaf. Do the same for a tenth of the leaf (10%): imagine 10 equally-sized divisions in the leaf. How big is each tenth? Then mentally cut each tenth in half to get 20 divisions of 5% leaf area. Finally, half of each of those units would be 2.5% leaf area. How big is that?
3. When it is time to do the actual herbivory estimate, one strategy that works well for contiguous blocks of damage is to use fractional thinking to zero in on the precise value, starting with larger fractions and gradually working your way down to smaller fractions—honing from a coarse estimate to a precise estimate. For example,
 - If ~12.5% of a leaf were damaged, then...

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- Mentally cut the leaf into quarters
 - See that less than a quarter (25%) is damaged
 - Mentally cut the quarter with damage in half, yielding eighths (12.5%)
 - See that the area damaged is equal to an eighth and record 12.5%
- If ~30% of a leaf were missing, then...
 - Mentally cut the leaf in half
 - See that less than half is damaged
 - Mentally cut the leaf into quarters
 - See that more than a quarter (25%) is damaged
 - Take mental note of the 25% damaged, and then focus on estimating how much more than that 25% is damaged
 - Mentally halve the quarter of the leaf with the excess damage above 25%, yielding eighths (12.5%)
 - See that the damage above 25% is a little less than half of one of those eighths, which means it's a little less than a sixteenth or 6.25%
 - 25% plus a little less than 6.25% comes close to 30%, record it!
4. If your leaf has more than one area of damage, try mentally consolidating each area of damage into one area and then estimate the size of that using the method above. Alternatively, if mental consolidation isn't working well, you can mentally divide the leaf into fractions that are as small as the smallest patch of herbivore damage. Then simply mentally tally the number of patches of that size that would be damaged.
 5. An acetate grid can be a very helpful tool. Some people use them to help guide their estimates on every leaf. Others use them occasionally for validating and calibrating estimates (e.g., on the first few leaves estimated each day). To make one, simply print out a grid cell on a transparency (make sure it's printer-friendly). Ian tends to print out several grid-sizes, and uses the size that has at least 20 grid cells for most leaves. Put the grid against the leaf. Count the number of grid cells with leaf (or where leaf should be) = T. Count the number of grid cells with damage = D. Percent damage is $100*D/T$. If you have 40 grid cells per leaf, then each grid cell will be 2.5%, a good target resolution. If you only have 20 grid cells per leaf, you can count in units of half grid cells to obtain a finer resolution. Ian likes the grid method, as he can do it while on a ladder. It has the downsides of being hard on oddly-shaped leaves (where most grid cell readings are exterior), only estimating damage with a resolution of $1/T$, and probably overestimating some damage types (like some beetle feeding) that may damage small parts of each grid cell.
 6. For complexly pinnate leaves (e.g., Apiaceae), it is probably best to divide the leaf into leaflets or pairs of leaflets, then follow the methods above.
 7. If damage is very high and very little leaf tissue remains, take a large and small leaf and compare the leaf base width, petiole and midrib size to compare. Use these comparisons to visually reconstruct the leaf, and deduce % damage from there
 8. If you have marginal damage on leaves with non-smooth margins (e.g., Figure 15.5): If you draw an entire margin a third of the way between the base of the margin teeth

15. Estimating Damage

and tip of the margin teeth, this approximately results in the same area measurement as if you had actually drawn in the margins—but it is easier/more accurate to imagine/draw a straight line than margin teeth

9. Piercing-sucking damage, when visible, should be mentally consolidated and estimated similarly to chewing damage. Be careful about confusing piercing-sucking damage and disease because they often look similar. If you are unsure, sleuth around your site to see if you can find the culprit in action. Sometimes it helps to find leaves that have damage at different stages of progression. This will let you reconstruct what older more necrotic tissue (and less discernible) might have looked like before it became so necrotic, perhaps inferring the cause of the damage. If this doesn't help, consult someone who may be able to or pick another species to survey.



Figure 15.5.: Leaf with both ‘interior’ and ‘leaf-margin’ damage.

10. For leaves with herbivore-built leaf shelters (rolls and ties), please carefully peer into or open shelters to estimate damaged area and count resident herbivores.
11. Through all of this, make sure you are correctly identifying what is herbivore damage versus disease versus physical damage. Please have a look at our A field guide to types of plant damage. We are trying to avoid damage caused by pathogens or abiotic stress. Before each survey, spend some time studying the range of damage types on plants in your population. Try to get a sense for what types of damage you might see during the survey. Sleuth out what damage types might just be physical damage (e.g., from wind). In this sleuthing, we have found it helpful to search for clues at both broad and fine scales. At broad scales, we search many plants across each site to see if we can find what is causing a particular type of damage. Often we will find the culprit, but only after a broad search. At fine scales, we use a hand lens to look closely at the damage. Often, a closer look at the damaged edges of a leaf reveals marks from insect mandibles. Tearing, in contrast, tends to be cleaner and more angular, often following even small leaf veins. Wind damage can manifest as browning. Look to at damaged spots to see if any tissue is actually missing. Only include necrotic tissue as herbivory if you are certain it is from herbivory. If you cannot be confident in your ability to tell apart physical and herbivore damage for a particular species or site, then please do not do the survey or consult someone who can help you.

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12. For internal feeding insects (e.g., hackberry psyllids, below):

- Count discrete units: count either the number of insects or the number of galls or mines. There are columns in the Template Datasheet for galls and mines.
- Mines should be included in percent damage and counted as discrete units.
- Galls should only be counted, not included in percent damage because galls are actually extra tissue! The removed tissue is internal and can't be seen.
- Keep an eye out for signs of stem-boring insects. Sometimes these can be counted.



Figure 15.6.: Internal feeding insects

15.4.1.2. How to make sure you're doing a good job

1. Be conscious that most people overestimate low levels of tissue damage (Johnson et al. 2016). Try to correct for this by being aware of this tendency, not rounding up at low levels of damage, and calibrating/validating estimates on leaves with low damage.
2. Invest time in practicing, calibrating, and validating estimates. Especially do this before collecting data, and continue to calibrate and validate regularly through data collection.
3. Standardize among observers you work with or have a single observer for all estimates.
4. Print out “[An illustrated guide to amounts of percent damage](#)”. Study it while practicing, and take it into the field with you as a reference. You can focus on the pages with leaves that are most similar in shape to leaves from your species.
5. Take our online herbivory estimation training quiz (in development, we will add the link here when it’s ready). This will help you assess your accuracy and precision and give you additional practice in estimating herbivory. We suggest re-taking this quiz once per week when doing surveys to refresh your memory.

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6. Finally, ground truth a subset of your damage estimates using a digital method. When doing this, please use 6 randomly selected plants in each survey. Do the survey as normal, but after visually estimating herbivory on each leaf in those plants use one of the two digital methods below to get a digital herbivory estimate (LeafByte or ImageJ). Make sure to record a unique identifier for each leaf to link visual and digital estimates.

- LeafByte: This is an app developed recently by scientists at Cornell (including our very own Zoe Getman-Pickering and Julie Davis). It goes on your iPhone and estimates damage of leaves that you photograph (it will tell you total leaf area, total damage area, and proportional damage). You can download the app and read instructions here. ‘BioLeaf’ is a similar app for Android phones.
- Scan leaves and estimate damage with Image J. For this, I usually collect leaves into a little bag. Once I’m back in the lab, I tape the leaves to a sheet of paper, and then use Image J (free software here) to estimate leaf damage. This is similar to LeafByte, but it takes longer.



Figure 15.7.: Three steps in measuring damage with Image J.

15.5. 4. Estimating Percent Damage Across the Whole Plant

The final damage assessment step is estimating percent damage across the whole plant, or as much of the plant as is feasible. We encourage you to strive to look across entire plants when estimating whole plant herbivory (unless your plants are > 2 m in height, in which case please follow the HerbVar Tree Protocol). For larger plants, there will be significant estimation error, but it is probably less than the error associated with subsampling, which could miss hotspots of herbivory within plants. Remember, the goal is just a visual estimate. You’d be surprised how quickly you can scan and integrate across a whole plant to estimate herbivory. However, we acknowledge this may not be feasible for large or complex plants; in those cases, please use one of the subsampling methods above and remember to record your methods and the size of your subsample. If you don’t feel that a whole-plant estimate is feasible, record the percent damage on 30 random leaves (see #3 above) and carefully

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record the total number of leaves on the plant and number of damaged leaves on the plant. The whole plant herbivory can be calculated from these values post hoc.

15.5.1. Tips for visually estimating damage across the whole plant:

Effective methods will vary a lot based on the size of plants, size of leaves, and architecture of plant. For smaller plants with a smaller number of leaves, you can quickly estimate damage on each leaf and combine leaf-level estimates into a plant-level estimate. If all leaves are similar in size, you can just average them. If leaves vary in size, you will need to take their relative sizes into account.

We often find it helpful to pick a reference leaf size on which to base mental calculations. Often it's convenient to pick the largest or smallest leaf, depending on whether you prefer scaling down or scaling up leaf level estimates.

An important tip to remember for speed is that when plants have more than ~9 leaves, leaves with low levels of damage will contribute very little to plant-level damage. For example, a leaf with 2% damage would only contribute 0.2% to overall plant damage on a plant with 10 similarly sized leaves. This means that you do not need to stress about these leaves. Of course, if every leaf on the plant has 2% damage, then this would be important to keep track off. Indeed, this is essentially what you need to pay attention to as you scan the whole plant. In our experience, most plants have skewed distributions of herbivory within plants, so it's all about paying attention to the proportion of leaves with insignificant and significant herbivory and the amount of herbivory on leaves with significant herbivory. But this isn't always the case, so look out for more even within-plant distributions. (Side note: we hope to get at this question with our herbivory estimates on the 10 random leaves).

For larger plants and plants with many small leaves, it is impractical to scan each individual leaf and mentally combine them (unless you are a mental math wizard!). In these cases, we still encourage you to scan the whole plant, but simply increase the grain size of your focus. For example, estimate herbivory at the scale of similarly sized branches of leaves. For plants with many, many small leaves, you may need to squint and look at similarly sized clumps of leaves. For example, people who work on conifers have a method for estimating herbivory on branches that involves looking up through a branch and seeing how much sky shows through.

Please let us know if you have additional tips, suggestions, or guidelines we can add to this document. And please let us know if anything is missing, confusing, or wrong! Have fun in the field and be safe.

! Important

If you're in an area with tick-borne diseases, don't forget to check for ticks after finishing fieldwork!

16. Reproductive Damage

16.1. Overview

This protocol aims to quantify damage by herbivores to reproductive structures of plants (i.e., flowers, fruits, and/or seeds). This is a supplement to the Primary Protocol (Chapter 14), which aims to randomly select and sample 30 plants, plus their nearest conspecific neighbor, within a population.

Objectives: The goal is to measure damage to reproductive structures on each plant within the surveyed population. Ideally, these measurements should be taken as supplemental data for the same individual plants (focal and neighbor plants) for which leaf damage was taken for the primary protocol because relating reproductive damage to leaf damage is a major research question. We recognize that this isn't always feasible, however, so reproductive damage measurements are welcome from plant populations without leaf damage measurements.

Datasheets: Record data in the “reproData” tab in the HerbVar template datasheet, which can be found here: Section 23.2.

16.2. Preparing to Sample

16.2.1. Select a species and population to survey

We are hoping to get broad taxonomic and geographic coverage of damage to reproductive structures. Therefore, any species could be surveyed. *However, to ensure that the data are comparable across sites/species/families/etc., the plants should have the following characteristics:*

1. At least half of the individuals at your site should possess reproductive structures. If most of the plants are in a vegetative stage, you probably won't be able to randomly sample and still get enough reproductive individuals to get a decent sample size. Ideally, >30 of the sampled plants will have reproductive damage data.
2. Enough of the individuals in the population should be in the same reproductive stage. If there is a mixture of flowering and fruiting plants within the population, it might be difficult to get a large enough sample size for one structure type. Additionally, different stages will likely be attacked by different herbivores.

16. Reproductive Damage

3. The plants should receive quantifiable damage to reproductive structures, as either percent area removed or a count of structures that are damaged or destroyed (see Section 16.5.2 on reproductive damage types below). Sometimes this damage is obvious (i.e., chewing to flower petals, damage to fleshy fruit tissue) while other times this damage is inconspicuous (i.e., damage to seeds inside fruits). It may be helpful to survey a few plants outside the focal population to look for these different types of damage before starting. Try opening a few fruits to check for internal damage.

If your plant does not meet these requirements, please skip measuring damage to reproductive organs. Or get in touch if you have questions.

16.3. Select a type of reproductive structure to quantify

Record the reproductive structure/unit you will survey, which could be any of the following: flower buds, flowers, inflorescences, immature fruits, mature fruits, infructescenses, or seeds.

Ideally, choose a reproductive structure for which there are at least 10 units per plant. We will still accept surveys for plants that have fewer than 10, especially if you can estimate a % damage for each structure (see below).

If possible, select a structure that will allow you to record counts of presence/absence of damage (method 1 in Section 16.5.2) AND a percent damage for each structure (method 2 in Section 16.5.2). This is ideal because it allows us to estimate both population-level (plant-to-plant) variation and plant-level (e.g. flower-to-flower) variation within plants, similar to the primary protocol for leaves. If this is not feasible it is also useful to record just the counts or just percent damage, whichever is feasible.

16.4. Establish a transect and select plants

Use the Primary Protocol (or an alternate protocol; see Chapter 13) to establish a transect, pick/calculate a quadrat radius, randomly select focal/nearest neighbor plants, and record covariates (plant size, life stage) for each plant (Primary Protocol Steps 14.1 - 14.3.4.b).

16.5. Record data for each plant

16.5.1. Record leaf damage

If possible, record leaf damage data for each plant (use Primary Protocol Step 14.3.4.c). Alternatively, if reproductive structures are best measured after the leaves senesce, you could mark the plants when recording leaf damage and return later to assess reproductive damage.

If you are going to record leaf herbivory and reproductive structure herbivory on the same individual plants, make sure to use the same plant IDs in both datasheets.

16.5.2. Record reproductive structure damage

For each plant you randomly select, record damage to reproductive structures in three ways:

1. **Presence/absence of damage** Record (A) the total number of reproductive structure on the plant, (B) the number of structures with any herbivory ($>0\%$), and (C) the number of structures that are inviable/destroyed (if possible to assess).
 - i) If the plant has 60 structures total, please record the true numbers.
 - ii) If the plant has >60 structures total, estimate the total number on the plant, then randomly (arbitrarily) choose 60 and record values for damage to those structures. Please also make sure to record that you took a subsample of 60 (see template datasheet).
 - iii) Optional: if the plant has multiple types of damage (e.g., chewing damage, piercing damage) or obvious pathogen damage, record the numbers of structures with different types of damage.
2. **Structure-level percent damage** Record the estimated percent damage (area removed) on 10 randomly (arbitrarily) chosen structures with herbivory damage ($> 0\%$ herbivory).
 - i) One estimate per structure (for a total of 10 estimates). If your plant has <10 structures with damage, record an estimate for each one.
 - ii) Please strive to sample in a way that selected structures will be representative of all reproductive structures on the plant (e.g., if fruits vary in size, sample different sizes in proportion to their occurrence on the plant).
 - iii) Note that all selected structures should have $> 0\%$ damage. Note also that measuring only damaged structures makes the data collected in step 1 (see above) vital in understanding per-plant damage variation.
3. **Plant-level percent damage** Record estimated percent damage on reproductive structures across the whole plant. For example, if a plant has 4 equally sized flowers and 2 of those flowers are 50% eaten, then whole plant has 25% herbivory. Take structure size into account when structures vary in size.
 - i) This estimate may not be feasible for some plant species with complex reproductive structures, if so it is fine to skip!

16.5.3. Continue using the Primary Protocol for each plant

Continue using the Primary Protocol to record any additional optional data on leaf pathogens and galls (14.3.4.e - 14.3.4.f), and the distance from the randomly selected plant to its nearest conspecific neighbor (14.3.4.g).

16.5.4. Repeat data collection for nearest conspecific neighbor of selected focal plant

As in the Primary Protocol, record all the same data as focal plant except nothing for neighbor's neighbor.

16.5.5. Continue selecting plants and recording data

Continue visiting the randomly selected points until 30 focal plants and 30 nearest neighbors have been surveyed.

16.6. Upload data in HerbVar xlsx datasheet

If you collect data on both leaf damage and reproductive structure damage in the same survey, be sure to include them in the same datasheet xlsx file (@-sec-datasheets) that you upload through the HV data portal.

16.7. Reproductive Survey Tips

- The first challenge is deciding if you can successfully survey reproductive damage. Start by exploring your plants. Casually examine at least 20 plants to get a sense of what interactions are happening at your site.
- Observe what type of damage is most common on the reproductive structures of plants in the population. Look for damage by insects that may chew on developing flowers (e.g. katydids, beetles), insects that bore into flower heads or seeds (e.g. larval weevils, leps, or flies), or true bugs that may probe/pierce the outside of fruits (looks like little black dots on the fruits), destroying the seeds inside. In many cases you will need to open the seed head/fruit to look for boring insects inside the seeds/fruit. Make sure to do any destructive sampling outside of your focal population.
- Look for signs of chewing damage inside the fruit, such as destroyed seeds and insect frass.
- Also, look for clipping damage by vertebrates that may have bitten off a whole flower or other structure.
- If enough of your plants (~50%) have the same reproductive structures and the damage is quantifiable, then you can do a reproductive damage survey!
- The best measurements of damage will depend on the type and extent of damage present. If the plant species experiences damage to multiple structures (e.g., petals, stamens, etc.), focus on the damage to the primary reproductive parts if it is not feasible to measure multiple structures.
- Consider whether you can confidently assess whether a reproductive structure (e.g. whole fruit, seed, flower) is likely destroyed or sterilized, such that it cannot successfully develop or germinate. For example, you may see entire flowers clipped, or seed-feeding insects that destroy the embryo. This is very important for understanding the potential fitness consequences of damage!
- There likely will need to be modifications for some species, but we trust collaborators to do the best they can in their systems while maintaining the overall spirit of the protocol. Keep in mind that the main goal is to capture the variability in damage rates among plants within a population, so you will want to choose a measure of flower/fruit/seed damage that best captures this variability.
- Make sure to record all of your decisions on the datasheet.

16.8. Examples of different types of damage to reproductive structures



Figure 16.1.: *Abronia umbellata* plant with petal chewing damage. For this plant, we would suggest using flowers as the reproductive unit. For method 1 (Presence/absence in Section 16.5.2), one would record the number damaged (3 damaged), the number destroyed (possibly 1), and the total number of flowers (13 total). For method 2 (Structure-level percent damage in Section 16.5.2), one would record the percent damage on damaged flowers ($1 = 60\%$, $2 = 10\%$, $3 = 100\%$). For method 3 (Plant-level percent damage in Section 16.5.2), one would estimate the percent damage on the whole plant (whole plant $\sim 10\%$ damage). This species typically has one or a few inflorescences, if it had multiple inflorescences the numbers for flowers could be summed across the inflorescences. Photo: Eric LoPresti.

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Figure 16.2.: *Lonicera x bella* fruit with chewing damage. These plants typically have large numbers of fruits per shrub. For method 1 (Presence/absence in Section 16.5.2), we could score up to 60 fruits for the presence/absence of damage. For the four fruits visible in the photo, 1 is damaged, 0 are destroyed, and 3 would not have damage (4 total). For method 2 (Structure-level percent damage in Section 16.5.2), we would estimate the percent damage on the fruit(s) with damage, for up to 10 fruits. The damaged fruit (2 bite marks) in this example would have approximately 10% damage. For method 3 (Plant-level percent damage in Section 16.5.2), we would estimate percent damage on the whole plant. For this example with the four visible fruits, whole plant damage would be approximately 2.5%. Photo: Susan Whitehead.

16. Reproductive Damage



Figure 16.3.: *Aquilegia shockleyi* with chewing damage to fruits (from *Heliothis phloxiphaga*). Here, if we choose fruits as our reproductive unit, in method 1 (Presence/absence in Section 16.5.2) we would count the number of damaged (2) and total (5) fruits. To determine if the fruits are destroyed/inviable, we would need to open the pods and examine the seeds. An entire fruit would only be counted as inviable if all of the seeds inside are inferred to be fatally damaged. In method 2 (Structure-level percent damage in Section 16.5.2) (optional), we could estimate the percent damage to each fruit as the percent of seeds that are destroyed. Photo: Eric LoPresti.



Figure 16.4.: *Vicia americana* seed pod on plant (left) and one removed and split open (right). Notice boring holes in the upper left seed and frass in the pod. In this example, choosing fruits as the reproductive unit, method 1 (Presence/absence in Section 16.5.2) would be to evaluate up to 60 pods on the plant for damage. Often damage is not visible to the outside of the pod, so each pod would need to be opened and then scored for damage. This may be a case where estimating a percent damage for each pod in method 2 (Structure-level percent damage in Section 16.5.2) would be difficult, since there are only a small number of seeds per pod. Alternatively, you could choose seeds as the reproductive unit. Each pod would need to be opened and the undamaged/damaged/inviable seeds counted together across all pods. Seeds that are completely consumed/missing should be counted as “inviable”, but be careful that you can distinguish a seed that was totally consumed versus an ovule that was not fertilized or developed. Photo: Phil Hahn.

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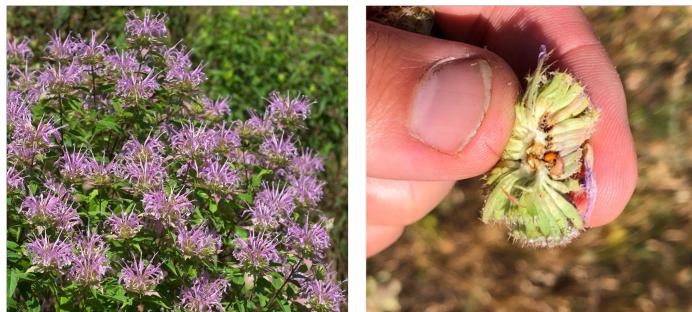


Figure 16.5.: *Monarda fistulosa* flowers on the plant (left) and one seed head removed and ripped open to reveal a lepidoptera larvae feeding on the developing seeds (right). This plant can produce a few (~3) to several dozen seed heads per plant. Each seed head contains dozens of floral tubes and each fertilized tube produces four seeds. In this example, we would suggest choosing inflorescence as the reproductive unit. In method 1 (Presence/absence in Section 16.5.2), we would open up to 60 seed heads to examine for presence/absence of damage to the seed heads. In method 2 (Structure-level percent damage in Section 16.5.2), for seed heads with damage, we would estimate the percentage of floral tubes that are damaged for each seed head. Alternatively, one could also choose flowers as the reproductive unit, but note that for this species, the process of counting individual tubes would be a time-consuming and tedious process, especially to do in the field. One might consider collecting damaged seed heads and bringing them to the lab for further evaluation. Photos: Phil Hahn.

17. Low Density/Abundance Plants

17.1. Overview

This protocol outlines three methods for surveying sites where the focal plant occurs at low density or low abundance. The Primary Protocol was designed to work for many plant species, growth forms, and contexts, but it requires sites with enough focal plants at a reasonably high density for efficient random sampling using our transect/sub-transect method. If the focal plants at your site are at very low density, then sampling them with our primary method will be very time-consuming due to the large distances between plants. If they are at low abundance, such that there are fewer than about ~90 plants in the site, then it does not make sense to draw a random sample of 30 plants + 30 neighbors from such a small population. If none of the methods below work well for your species and site, we encourage you to think of a comparable alternative. Feel free to get in touch if you have questions. Regardless of what you decide, please make sure to carefully document your methods.

We provide three separate protocols for surveying sites with low density and/or abundance of focal plants:

1. Comprehensive Patch Census.
2. Walking Transect.
3. Comprehensive Census of Subset.

17.2. Option 1: Comprehensive Patch Census

The best method, when feasible, is to census all of the individuals in a patch. This will work when there is a well-defined patch with a reasonable number of plants (e.g., < ~90). If this is possible, it is better in many ways than the Primary Protocol because it describes the whole distribution of herbivory at the site—there's no risk of missing the tails of the distribution if every plant has been included! Also, depending on the context, this could be faster than our Primary Protocol because setting up transects and quadrats to randomly sample plants is time consuming. So comprehensively examining all of a patch's 90 plants, for example, might be faster than randomly sampling 60 plants (30 focal plants and their 30 neighbors) from a larger population. For this method, we stress that you should strive to survey every plant.

17.2.1. Record spatial information via one of two methods

Option A. Record GPS position of each plant. If you do this, you will not need to record nearest neighbor information because we can reconstruct it (and more!) easily from the geographic coordinates. This of course means that you will need a GPS sensitive enough to differentiate the locations of your plants. If your plants are on average more than 2-3 m apart, then almost any modern GPS will be precise enough. If, however, your plants average less than ~1 m apart, then you would need a very precise GPS to describe the relative locations accurately. If you don't have such a precise GPS (or if you don't like how slow a precise GPS can be), we recommend the second method.

Option B: Relative spatial coordinates: You can measure the relative coordinates of your plants using two tape measures or a tape measure and a meter stick. This sounds similar to the primary protocol but it's much quicker because you're not using the tapes to select plants, just to record their locations.

1. Lay a tape measure through your patch. For each plant, record spatial coordinates as how far along the tape measure and how far from the tape measure. You can situate the tape either along the edge of the patch or through the middle of the patch.
2. If your tape measure is through the middle of the patch, remember to record the distance left of the tape as negative and right of the tape as positive. The start of your tape will have the coordinate (0,0).
3. After recording this information, follow the Primary Protocol as closely as possible

17.2.2. Other useful information

Record `popDiameter1` and `popDiameter2` as the approximate extents of your patch/census area

For focal plant percent cover (`focalPlantCover`) and non-focal plant percent cover (`otherPlantCover`), please follow the Primary Protocol methods for estimating population density and calculating a quadrat radius size, if feasible. You can then center a quadrat on each focal plant in the census to define an area around each focal plant for recording focal and non-focal percent cover, as well as the number of focal plants in quadrat (`numPlantsinQuad`).

In comprehensive surveys the ~60 plants will all be focal plants and there is no random selection; hence the nearest neighbors (all the ".1" plant IDs in datasheet template) become focal plants. As described above, a quadrat is centered around each plant and the three quadrat-level variables (`focalPlantCover`, `otherPlantCover`, and `numPlantsinQuad`) are recorded for each plant. The nearest neighbor distance (`NNdist`) is still recorded, but since that neighbor is treated as a focal plant, please record the unique `plantID` of that nearest neighbor as well (e.g. add `NNplantID` column as needed in datasheet).

If you cannot estimate population density (e.g., because your species is too sparse), then please pick an arbitrary quadrat radius. You can use that to define an area around each focal plant in your census for estimating percent cover variables. A 1-m radius might be

a good choice for many plants, but go bigger for bigger plants. Remember to record your choice!

17.3. Option 2: Walking Transect

Another alternative if you have widely dispersed plants that do not form a well-defined patch (or the patch is too large for a comprehensive search) is a walking transect.

1. Randomly pick distances (e.g., paces) along a transect and from a transect.
2. Pace out the distance along the transect, then turn orthogonally to pace out the distance from the transect.
3. Survey the closest plant within some reasonable distance (if no plant is reasonably close, then go back to transect and keep going).
4. Repeat until you have 30 plants and 30 neighbors.

This is similar to the Primary Protocol except pacing (rather than measuring with a tape) can make large areas more feasible to survey. Consider recording spatial coordinates for each plant, especially if plants are far from your randomly identified points. And try to survey neighbors for each plant.

17.4. Option 3: Comprehensive Census of Subset

This method is similar to the comprehensive census of a patch (#1 above), but it applies when there is no well-defined patch and individuals are widely dispersed over a large area.

There are two ways to do this, but for both of these methods record spatial

coordinates for each plant as described in Option #1 above.

Option A - Comprehensive survey of all plants along a transect: With this method, you are doing a comprehensive survey of a linear subset of the whole population.

Start by randomly picking a transect starting point and direction. Walk the transect and survey every plant that crosses your path *or* every plant within a reasonable distance of your path (e.g., 2 m). Keep going until you get at least 60 plants.

Option B: Comprehensive survey radiating out from a random starting point within a population. With this method, you are doing a comprehensive survey of a roughly circular (or blobby) area within the whole population.

Explore outwards from your random starting point, surveying every plant you encounter until you get to at least 60 plants. *Note that we do not recommend doing this unless your plants truly are all widely dispersed.* If your plants are close together there could be high spatial autocorrelation such that you fail to capture the range of herbivory levels in the population. Of course, this is always a risk; it's just especially acute when the sampling extent is an arbitrary area rather than a biologically significant "patch".

18. Cacti & Succulents

18.1. Overview

This document discusses issues relevant for quantifying herbivory on cacti and outlines a hopefully widely applicable protocol for doing so. The protocol is designed for cacti that have many jointed segments, but we also discuss ways to modify the protocol for other architectural types. Although we focus on cacti, we think this document will also be helpful for other succulents. Please share feedback, particularly ways we can make this widely useful.

Unique Context: With regard to quantifying herbivore damage, cacti are special: (a) they are architecturally unique, (b) architecturally distinct from each other, and (c) much of the herbivory is surficial (there are few “edges” to bite!) and (d) since units are not lost, damage can persist for decades. Any census method needs to take these factors into account.

18.2. Sampling Issues within Architectural Categories

Cacti can be thought to consist of one or more (usually spiny) tubes with flowers usually located at the tip of the tube – that’s what they have in common – with these tubes having a diversity of spatial relationships to each other – that’s what makes them different from each other. *There are at least five categories, each with its own herbivory sampling issues.*

1. **Single, unbranched tube stuck in the ground** (e.g., in the American Southwest, a barrel or a pincushion cactus). As they age, they get taller and wider, but they never branch or clone. If there is a cluster, they are genetically different from each other (I am almost sure, JB).

Entire structure should be scrutinized for herbivore damage. When these cacti form a cluster, they should be categorized as multiple individuals rather than as a single individual.

2. **Set of unbranched tubes connected underground** (e.g., a hedgehog cactus (small), or a senita or organ pipe cactus (large)). They add units as they age.

Either the whole thing can be scrutinized for damage, or a subset of units could be (please make a note of which path you chose). If there are not that many units, full sampling is possible. However, some of these cacti get very tall. Since much damage seems to accrue at tube tips, one really should look at the entire length.

3. **Tube that starts to branch above the ground** as it ages (e.g., a saguaro cactus).

Same method as #2 (again, please make a note of whether you examined the whole plant or a subset of units).

4. **Large set of tubes connected at distinct joints** (e.g., a cholla cactus). New tubes are added as the plant ages.

Subsampling within individuals will usually be necessary because individuals often have many tubes. Our protocol below, which is focused on cacti with many joints, describes a method for subsampling up to 20 joints per plant.

5. **Large set of tubes connected at distinct joints but flattened into pancakes** (e.g., a prickly pear cactus). New tubes are added as the plant ages.

Same method as #4.

18.3. Expected Types of Herbivory

1. **Bites that remove chunks of flesh.** This is quite obvious for the fifth category of cacti (flattened pads), because the pads have smooth edges that will be disrupted by this type of herbivory. So if this category is being surveyed, special attention should be paid to the edges of pads. I am not sure if it will be evident, or at least common, for any other category: only this category consists of units with “edges”. I suspect that other cactus-feeders that take out chunks of flesh concentrate on the youngest tissue (new units and the tips of existing units), and this suggests important sampling rules: young units should be sampled, as well as tips of existing units (which, unfortunately, might be very high in the air). Some large beetles burrow into cactus flesh, but (based on my knowledge of barrel cactus) these individuals rapidly die, so this sort of damage is unlikely to persist
2. **Scarring of the surface of the cactus.** It can be very hard to know what causes this – some of this damage may be attributable to herbivores, but some might be fungal or bacterial attack. It is worth taking photos of the damage and trying to figure out the culprit. Damage left by various small herbivores on various cactus species has been described in the literature and it may be worth making a photo album for later identification.
3. **Colonies of sucking insects.** In particular, cochineal bugs live in colonies and are exciting to see. They are covered with a messy white wax. At least in the desert Southwest, cochineal are primarily found on introduced *Opuntia ficus-indica*. However, there are small colonies on *Opuntia engelmannii* as well that should be watched out for.

18.4. Additional Considerations

1. **Some genera have species with extrafloral nectaries (EFNs).** Most (not all) barrel cacti have them, and I believe all columnar cacti (senita, organ pipe, saguaro),

prickly pear, and cholla do. On the other hand, I don't know of any hedgehog or pincushion cacti that have them. Ant attraction to extrafloral nectaries may reduce herbivore attack, though field evidence for this has varied across cactus species, and ants are often surprisingly rare. Most EFN-bearing cacti only secrete nectar when there is new vegetative growth, buds, flowers, and early fruit present, but some (such as the fishhook barrel cactus abundant in Tucson) secrete it year-round.

2. It seems likely that the **newest, tenderest units are particularly likely to be attacked** (particularly in categories 4 and 5 cacti).
3. The **buds and young fruits of some cacti get very heavily attacked**, and these should be included by counting damaged and undamaged units and recording the data separately (see Reproductive Damage Protocol).

18.5. Protocol

We designed this with prickly pear (*Opuntia* spp.) in mind, but it should work essentially the same way for other cacti with many jointed tubes such as cholla (*Cylindropuntia* spp.). Modifications will be necessary for some cacti. *The gist of the protocol involves following the Primary Protocol except for a subsampling of leaves and reproductive units (if present) within plants.* We suggest taking both this protocol and the Primary Protocol with you in the field.

18.5.1. Pre-Census Tasks

1. Pick a species to census.
2. Choose a site, ideally with at least 90 well-defined individuals that you can randomly sample using the HerbVar Primary Protocol. If your site has fewer than ~90 individuals or has very widely spaced individuals, we suggest following methods from our document on Surveying low-density/low-abundance sites.
3. Decide on a maximum number of pads per plant to census.
4. We recommend focusing only on young cactus pads. But you should decide if this will do a good job representing the plant-herbivore interaction and distribution of herbivory for your species. By young pads we mean those that are final joints, i.e., that don't have another pad growing out of them. Problems with older pads include:
 - a. Older pads can be many years old, thus integrating herbivory over a much longer time than happens for other plant species in HerbVar (few plants hold leaves as long as cacti hold their pads)
 - b. Practically, it's very hard to determine and quantify what is herbivory vs physical damage on older pads
 - c. Physically, it can be hard and dangerous to access older pads on spiny plants!
 - d. If you think focusing on young pads will not be good for your species, then please modify the protocol to include older pads. Take detailed notes.

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5. Ideally, investigate the major types of damage you may see, potentially making a cheat sheet of photos.
6. See how long the protocol outlined below would take, then modify as necessary. Be sure to take detailed notes on any modifications made to the protocol.

18.5.2. Census

1. Record site characteristics (e.g., date, site, plant ID, etc.)
2. Review above discussion of architectural categories of cacti/succulents and decide how you will define an individual plant. Past populations we surveyed had many very large clumps of pads that were almost certainly one plant individual, though not all connections were visible aboveground. In most cases, the clumps were discrete enough that we were confident each clump was one individual with below ground connections.
3. If you have a site with >90 plant individuals, follow the Primary Protocol from the beginning until you have your first plant for herbivory estimation. Briefly:
 - a. Pick transect and subtransect distances that will encompass your site and lay the transect through the site.
 - b. Estimate the density of plants in the population.
 - c. Use the estimated plant density to calculate a quadrat radius to use for the survey.
 - d. Randomly generate x,y points, visit them, and set up a quadrat centered on each random point, selecting 1 plant randomly within each quadrat. See the Primary Protocol for more detail.
4. Once the first plant is selected, survey it for herbivory. For vegetative herbivory, we recommend focusing only on terminal pads (see #4 above). Terminal pads are those at the end of a branching structure, without another pad growing out of them. Also, focus only on the visible surfaces of pads because moving spiny pads safely is difficult and time consuming! **Record herbivory from all organisms in one column and herbivory you are certain was just from insects in a second column.** We found it was difficult to distinguish insect herbivory from vertebrate herbivory, so we usually recorded “totalHerb”. Occasionally it was clear some herbivory was just from insects, so then we used the “insectHerb” column to indicate what percent was definitely from insects. **There are 3-4 herbivory estimation steps, depending if your plant has reproductive organs:**
 - a. Quickly scan all of the terminal pads across the entire plant and visually estimate percent herbivory. This is a quick estimate, but it’s important to scan the whole plant because herbivory can be patchy within plants.
 - b. Randomly select 20 of the terminal pads. Record the number of pads you examined and the number with any herbivory present. If there are fewer than 20 pads on the plant, then do all pads.

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- c. Randomly select 10 terminal pads, and record a visual estimate of the percent herbivory on each pad, resulting in 10 numbers (one number for each of the 10 pads). We estimated percent herbivory as surface area removed on the visible faces of paddles (as opposed to volume or doubling the area for holes that went entirely through a paddle).
 - d. If your plant has reproductive organs, please randomly (arbitrarily) select up to 20 units of one type (e.g., flowers or fruits) and record the number of units you examined and the number that were damaged (>0.5%). Please see the Reproductive Damage Protocol for more information. You will need to add columns to the HerbVar Template Datasheet to accommodate this.
5. Note presence of pathogens.

19. Trees

19.1. Overview

Mature trees, though harder to study than smaller plants, are a key plant growth-form that could have their own characteristic patterns of interactions with herbivores. Therefore, we want to include enough surveys of mature trees in HerbVar's global sampling to allow us to compare patterns between trees and other growth forms. It is also important to include mature trees because there may be major shifts in tree-herbivore interactions with tree ontogeny, from seedling to sapling and sapling to adult. *Here we provide three protocols:*

1. 'Tree Seedlings & Saplings' Protocol
2. 'Mature Trees' Protocol
3. Protocol for selecting leaves for herbivory estimates

Objectives: Provide a protocol for sampling mature trees. Collaborators who do not have a special interest in working with mature trees should restrict their surveys to individuals 2 m height (seedlings and saplings). That is, survey tree species, but focus on seedlings and saplings. Seedling-sapling surveys won't be representative of all the individuals in a population of a tree species, but these are key stages in tree ontogeny—perhaps the stages in which herbivory is most influential. We are taking a two pronged approach to including tree species in HerbVar.

19.2. 'Tree Seedlings & Saplings' Protocol

Follow the Primary Protocol. This includes (but isn't limited to) the following data:

1. Leaf-level percent herbivory estimates for 10 randomly selected leaves
2. Counts of presence/absence of herbivory for up to 60 leaves per plant
3. A whole-plant visual estimate of herbivory
4. And of course please record the number of galls, mines, and other discrete damage types from sessile herbivores

Please note in the metadata that you surveyed only immature individuals (2 m) at your site. Such a note can be complemented by recording the height of the individuals in the `plantSize` columns

19.3. ‘Mature Trees’ Protocol

Follow the Primary Protocol & Site Selection Protocol to pick a site, set up a transect, and randomly select focal trees or quadrats.

Many tree species cover huge geographic areas, making it unreasonable to survey an entire “population” or define a discrete study “site.” If you are working with a widespread species, it is fine to choose a “representative” study area, which actually might just be a small part of a large stand of trees.

Once you have selected a representative study area, you will need to **randomly select 30 trees to survey** (plus their nearest conspecific neighbors). There are many ways to do this. **Here are three methods in somewhat decreasing order of amount of work and rigor:**

1. **Follow the Primary Protocol exactly**, establishing a transect, selecting 30 points randomly (distance along main transect and distance from main transect), and using a circular quadrat at each point to randomly select 1 individual of the tree species within the quadrat (as in the Primary Protocol). Survey each selected tree and its nearest neighbor.
2. **Follow the Primary Protocol except skip the circular quadrat step**, which could need to be prohibitively large in some tree populations: Establish a transect and randomly select 30 points (distance along main transect and distance from main transect). Then select and survey the individual nearest to each random point (plus nearest neighbor).
3. **For trees that are at low density or low abundance, use the Low Density Protocol (Chapter 17) to select trees.** For low-abundance plants, we recommend surveying every plant within some area. Take GPS coordinates for each plant. Try to get as close to 60 plants as possible. If you are taking GPS coordinates for each plant, then you do not need to measure distances to nearest neighbors because we can measure spatial relationships using the GPS data.

However you select trees, please make sure to take detailed notes on what you did. Note that some of the trees you select may be seedlings or saplings. We recommend doing whichever individual you randomly select, regardless of its age. This should yield a representative sample of all individuals at the site, across age classes.

Randomly select 30 leaves for quantitative estimates of percent herbivory on each of the 30 leaves.

1. See Primary Protocol and Damage Estimation Training Document for guidelines on quantifying percent herbivory per leaf.
2. Please also record the number of galls, mines, and other discrete damage types. Note that mines should be included both in percent damage (because they represent damaged surface area) and as counts.
3. Randomly select an additional 30 leaves to score for presence/absence of herbivory. Record the number out of 30 with herbivory.

Do not worry about estimating herbivory at the whole-plant scale for mature trees; we will estimate this using the 30 presence/absence leaves and the 30 percent herbivory leaves

19.4. Protocol for selecting leaves for herbivory estimates

Trees... are tall, and we will not be able to reach top branches. We will therefore focus on low branches that can be reached from the ground with a pole pruner, and sample from multiple places around the circumference (see Figure 19.1). We provide some guidelines below, but you should choose an approach that makes sense for you and your species. *Remember that we are trying to acquire a random subsample of all leaves on the tree; this means avoiding any preference for/against particular leaves (e.g., young vs old).* Ideally, leaves will be sampled in proportion to their frequency on the tree. **Here are two alternate methods for selecting random (or at least haphazard) leaves:**

1. **The easiest method:** if it would work for your trees, is to close your eyes, point at the tree, open your eyes, and take the leaf you were pointing at (“Ian’s nose pointing method” in Chapter 15).
2. **Perhaps the most rigorous but most time-consuming method:** is to haphazardly strip several times as many leaves as you need (e.g., >200 leaves). Place leaves individually into a large bag. Mix them. Close your eyes and draw 30 leaves for percent herbivory and 30 leaves for presence/absence of herbivory.



Figure 19.1.: Focus on low branches that can be reached from the ground with a pole pruner, and sample from multiple places around the circumference

20. Rhizomatous Geophytes

20.1. Overview

In semi-arid and arid climates, a considerably large number of plant species are rhizomatous geophytes. Their major characteristic is that they grow as patches of individuals, forming either dense (phalanx) or sparse (guerrilla) mats of individual ramets, each visible as a single leaf fan, and all connected through below-ground rhizomes and/or above-ground stolons into one plant (genet) Herben and Klimešová (2020). The extent of clonal growth defines the spread of the genet, and is on a continuous scale of density (Vallejo-Marín et al. 2010). See Figure 20.1 for examples of two density levels of genets in irises.

Clonal plants present an interesting challenge and opportunity within the HerbVar Network. From a question-based perspective, we may be able to compare patterns of herbivory variability between clonal vs non-clonal plant species. These different modes of reproduction may confer different levels of genetic and phenotypic diversity within plant populations (Sapir and Shmida 2002, Wilson et al. 2016), which could affect patterns of herbivory. However, from a practical perspective, quantifying herbivory among plant ‘individuals’ is a challenge in these systems (i.e., what constitutes an ‘individual’?).

Objectives: Provide a protocol for surveying herbivory on a rhizomatous plant species that meets two conditions: (1) it is feasible to determine what constitutes a genet by examining rhizomatous connections, and (2) genets are small enough at your study site that you could feasibly survey 30 genets and their nearest neighbors and estimate herbivory on each genet.

20.2. Rhizomatous Geophytes Protocol

When first starting this for a new species or at a new site, we suggest spending time investigating what constitutes a genet. Follow rhizome connections from ramet to ramet to get a sense of what a single genet looks like before following the rest of this protocol.

1. Follow the Primary Protocol & Site Selection Protocol to pick a site, set up a transect, and randomly select focal quadrats’ locations
2. Calculate a custom radius for circular quadrats.
 - a. Estimate mean density of genets per square meter by counting the number of plants in 1 m² at 10 random locations within the site.

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- b. If genet area (clone/genet diameter) is >1 m and/or distances between genets are apparently irregular (that is, secondary dispersion of plants within population is patchy), count the number of genets in 1 m² every 5 meters along a 50 m transect.
3. If a quadrat has 0 focal plants, record a 0 and move to the next quadrat. If a quadrat has >0 focal plants, randomly choose 1 of the genets to survey and record the following data:
 - a. Genet life stage: seedling, vegetative, reproductive.
 - b. Genet size: measured as the height of the tallest leaf for plants in vegetative stage, or height of the taller flower for plants in reproductive stage. Record which metric you used in the `plantSizeMetric` column
 - c. Herbivore damage in one of 3 ways:
 - d. Total number of leaf fans (ramets). For genets with >100 ramets, write “100” and make a note that your estimate was capped at 100.
 - ii. Estimated percent damage across the whole genet. Visually scan all the green areas of all ramets and all leaves, and estimate the percentage of damage.
 - iii. If the plant has <10 ramets, sample all ramets. From each chosen ramet, pick the 2nd or 3rd leaf from top and estimate percent herbivory. These leaves are putatively in the same developmental stage and are the same age, thus exposed to herbivory for equal time.
 - iv. **Note:** the tip of the leaf may be dry due to climate fluctuations in the arid regions. This area of dry leaf counts as leaf area, but not as herbivory damage.
4. Record the same data for the first nearest conspecific neighbor (of a different genet) that you recorded for the focal plant.
5. Continue visiting randomly select points until 30 focal genets and 30 nearest neighbor genets have been surveyed

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Figure 20.1.: Top left: Dense (“phalanx type”) genet of *Iris atrofusca*; Top right: Sparse (“guerrilla type”) genet of *Iris bismarckiana*; Bottom left: Compact rhizome of *Iris atrofusca* (this one has ~4 leaf-fan ramets); Bottom right: Stolons connecting ramets of *Iris bismarckiana*.

21. Herbivores, Mines, & Galls

21.1. Overview

Though a lower priority than the damage data, these data will permit us to pilot some more mechanistic questions about the distribution of herbivory (e.g. spatial aggregation of herbivores). So far, observers have been recording as much herbivore data as they can via a quick visual survey; however, this may not be feasible for all observers or systems.

! Important

For all plants, record the number of leaf mines and galls on the entire plant. If there are too many to count individually, please estimate (for example, by counting the number present on some module of the plant [e.g., a branch] and multiply by the number of modules).

Separate from counting mines and galls, please also collect insect herbivore data if you are confident in insect ID (see below for specifics).

21.2. Should you sample “core” herbivores?

Please use the following questions to help you decide whether to sample “core” herbivores.

1. Are you comfortable distinguishing the following 5 groups of herbivores?
If not, prioritize another herbivory survey (see the Primary Protocol).

- Grasshoppers/crickets/katydid (Orthoptera).
- Caterpillar-like larvae (i.e., eruciform larvae). **Note:** this includes moth/butterfly caterpillars, sawfly (Hymenoptera: Symphyta) larvae, and some beetle larvae but does not include larval true flies (i.e., maggots)
- Aphids (Aphididae)
- Hoppers (Hemiptera: Auchenorrhyncha). This includes planthoppers (Fulgoromorpha), leafhoppers (Cicadellidae/Cercopidae), treehoppers (Membracidae), & cicadas (Cicadidae). If you are confident, you may also identify non-“hopper” Auchenorrhynchs in the column provided in the template Excel file
- Non-Aphid Sternorrhynchs. This includes whiteflies, scale insects, and mealybugs

2. **Are you confident that you can visually detect the herbivores on the selected plant species**, considering the complexity of plant structure? If not, prioritize another herbivory survey (see the Primary Protocol). If you have the ability to sample herbivores in another way (e.g., a beat-sheet) and feel excited about this, feel free – but be judicious of the added time required for sorting through a loaded beat-sheet!
3. **Could you do another herbivory survey with the time required to conduct an herbivore survey?** If yes, prioritize another herbivory survey (see the Primary Protocol). If not, please collect herbivore data!

21.3. Protocol

21.3.1. Sampling insects beyond “core” herbivores.

In an effort to standardize the insect data we have included 5 groupings to use for tallying herbivores. This is to avoid counting insects which may be predatory, rather than herbivorous (e.g., “true bugs”). ***Please prioritize counting herbivores belonging to the 5 aforementioned groups*** (see visual guide below if needed).

Please indicate whether you are recording herbivores as a count or as presence/absence data (see `insectUnit` in the `herbivoreData` tab of the template Excel file)

For both core and non-core insects, please count herbivorous insects whether or not they are actively feeding. You are welcome to make a note of their behavior in the “notes” column but all potential herbivores on the plant should be included in your survey

If you have more intimate knowledge of insect herbivores (e.g., can distinguish herbivorous true bugs from predatory), please add columns for these other insects in the `herbivoreData` tab of the template Excel file. To facilitate this, we have added `beetleHerbivore`, `thysanopteraHerbivore`, `gastropod`, `stemBorers`, and `heteropteraHerbivore` to the template digital datasheet but you are welcome to add other columns as needed. We also recognize that herbivore surveys may differ dramatically among sampling sites and have modified our printable datasheet for this survey to include an `Insect ID` column rather than predefined columns

Please continue to record the 5 required insects (even when there are none please put a zero). While mines/galls are recorded in the Primary Protocol, mine-/gall-forming insects should be counted here if you have the time and identification ability to search within galls/mines for insects

21.4. Herbivore Guide

21.4.1. Mines & Galls Visual Guide

How do you count serpentine mines? It’s a confusing picture but in that way is more likely something that would be seen in the field! One of our gall gurus (Eric LoPresti) thinks this is probably two mines. He says, “the one that terminates at the top in a blotch and

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Figure 21.1.: Leaf mines – Linear/Serpentine. Left/right = single mine. Center = multiple mines

the one that terminates at the bottom center in a wider figure 8 - looks confusing since the bottom one doubled back, making a weird hanging trail. But you can tell that it is a single mine, since there is no nearby really thin trail where it starts. The intermediate width mine on the right is odd - whether it was aborted/eaten or doubled back is not obvious to me, however, I suspect it is the latter, as I only see two really thin sections, both on the upper half, which indicates a start and a very small caterpillar.”

This can get confusing, but do your best. Each count doesn't have to be exactly right; we should still be able to get a representative count of the distribution of damage & mine frequencies. If in doubt with these serpentine mines, standardize by counting only the blotchy/expanded mine ends; this will ignore (but in a consistent way) aborted or re-started mines. Make a note of this in the data if you choose this method.

21.4.2. Leaf Mines – Blotch Mines



Figure 21.2.: Examples of multiple blotch mines on single leaves

21.4.3. Galls – Leaf Galls



Figure 21.3.: Leaf Galls

21.4.4. Galls – Stem/Branch Galls

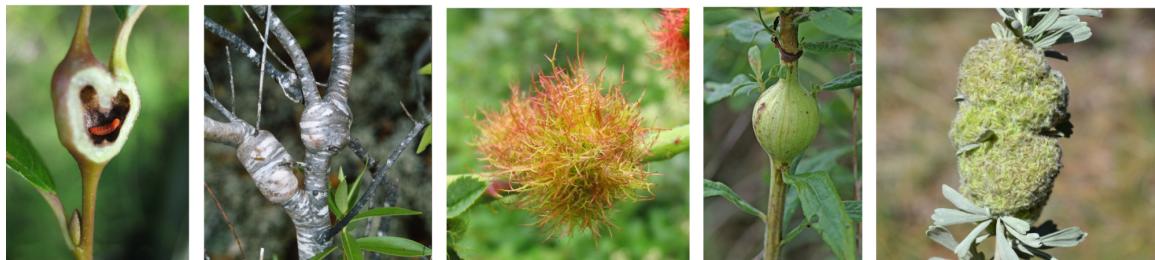


Figure 21.4.: Stem/Branch Galls

21.5. Insect Herbivore ID Visual Guide

Some groups of insects (e.g. Hemipterans, Coleopterans) include predatory, herbivorous, and omnivorous species - and it can be challenging to tell the two groups apart. Other groups are more certain to be herbivores. Use this visual guide to identify insects within the five core groups.

21.5.1. Grasshoppers, crickets, & katydids (Orthoptera)

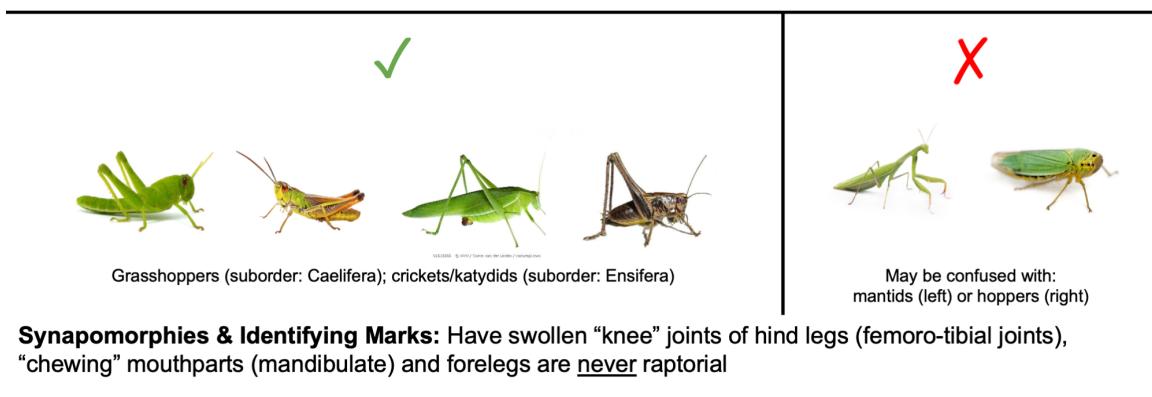
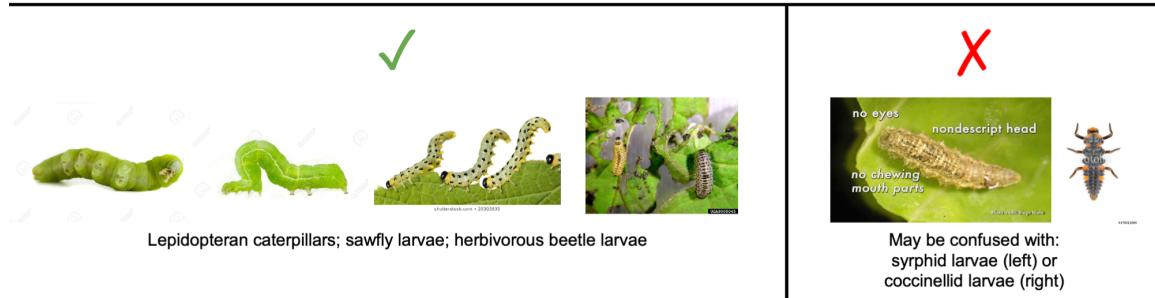


Figure 21.5.: Grasshoppers, crickets, & Katydids.

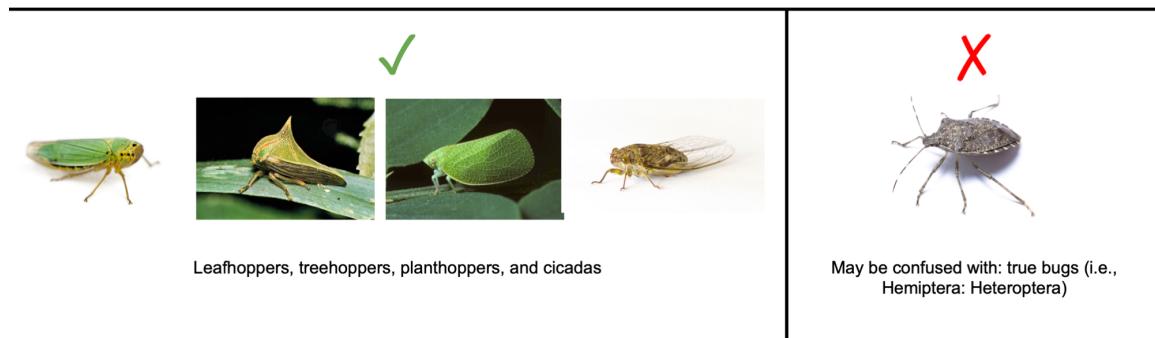


Synapomorphies & Identifying Marks: Have cylindrical bodies with a well-developed head capsule. Have both true (thoracic) legs and abdominal prolegs. Specimens lacking legs entirely or with only six legs do not belong in this group.

Figure 21.6.: Larval forms of caterpillar-like herbivores.

21.5.2. Caterpillar-like (larval forms ONLY)

21.5.3. Hoppers (Hemiptera: Auchenorrhyncha)



Synapomorphies & Identifying Marks: Have piercing/sucking mouthparts with “beak” seeming to originate from the neck. Wings or dorsal exoskeleton sometimes modified into leathery covering. Antennae bristle-like (aristate) and short originating beneath eyes

21.5.4. Aphids (Hemiptera: Aphididae)

21.5.5. Non-Aphid Sternorrhynchans (whiteflies, mealybugs, & scale insects)

21. Herbivores, Mines, & Galls

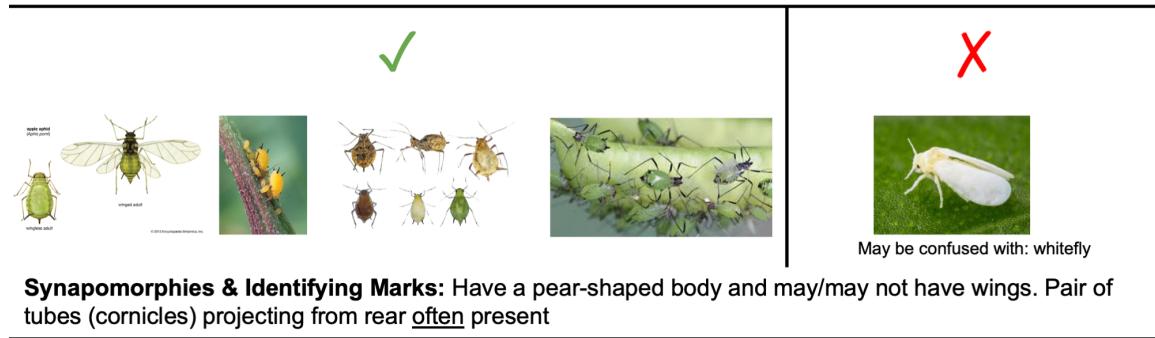


Figure 21.7.: Aphids.

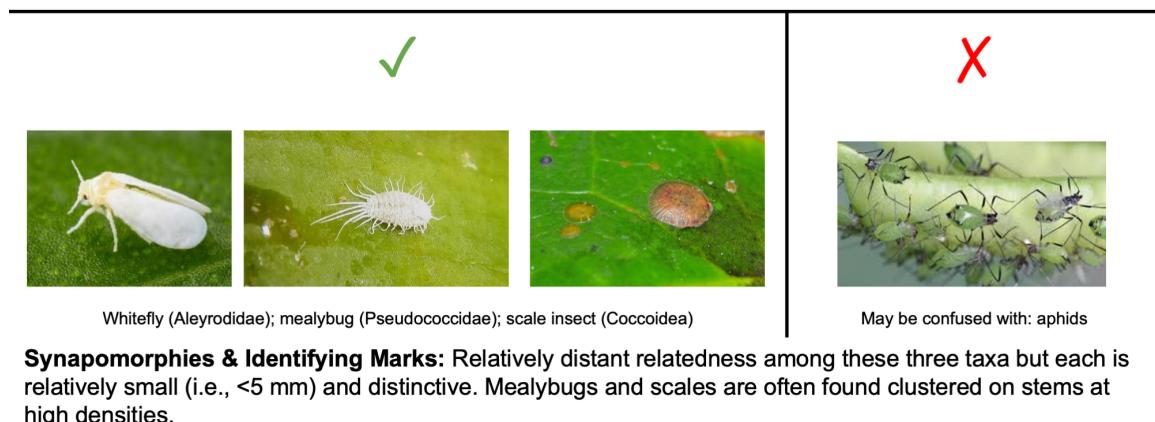


Figure 21.8.: Whiteflies, Mealybugs, & Scale insects

References

- Boland, M. R., K. J. Karczewski, and N. P. Tatonetti. 2017. [Ten Simple Rules to Enable Multi-site Collaborations through Data Sharing](#). PLOS Computational Biology 13:e1005278.
- Braga, P. H. P., K. Hébert, E. J. Hudgins, E. R. Scott, B. P. M. Edwards, L. L. Sánchez Reyes, M. J. Grainger, V. Foroughirad, F. Hillemann, A. D. Binley, C. B. Brookson, K. M. Gaynor, S. Shafei Sabet, A. Güncan, H. Weierbach, D. G. E. Gomes, and R. Crystal-Ornelas. 2023. [Not just for programmers: How GitHub can accelerate collaborative and reproducible research in ecology and evolution](#). Methods in Ecology and Evolution 14:1364–1380.
- Champieux, R., A. Solomonides, M. Conte, S. Rojevsky, J. Phuong, D. A. Dorr, E. Zampino, A. Wilcox, M. B. Carson, and K. Holmes. 2023. [Ten simple rules for organizations to support research data sharing](#). PLOS Computational Biology 19:e1011136.
- Goodman, A., A. Pepe, A. W. Blocker, C. L. Borgman, K. Cranmer, M. Crosas, R. D. Stefano, Y. Gil, P. Groth, M. Hedstrom, D. W. Hogg, V. Kashyap, A. Mahabal, A. Siemiginowska, and A. Slavkovic. 2014. [Ten Simple Rules for the Care and Feeding of Scientific Data](#). PLOS Computational Biology 10:e1003542.
- Harper, J. L. 1977. Population biology of plants. Academic Press.
- Herben, T., and J. Klimešová. 2020. [Evolution of clonal growth forms in angiosperms](#). New Phytologist 225:999–1010.
- Johnson, M. T. J., J. A. Bertrand, and M. M. Turcotte. 2016. [Precision and accuracy in quantifying herbivory](#). Ecological Entomology 41:112–121.
- Kim, A. Y., V. Herrmann, R. Barreto, B. Calkins, E. Gonzalez-Akre, D. J. Johnson, J. A. Jordan, L. Magee, I. R. McGregor, N. Montero, K. Novak, T. Rogers, J. Shue, and K. J. Anderson-Teixeira. 2022. [Implementing GitHub Actions continuous integration to reduce error rates in ecological data collection](#). Methods in Ecology and Evolution 13:2572–2585.
- Kvålsseth, T. O. 2017. [An alternative measure of ordinal association as a value-validity correction of the Goodman–Kruskal gamma](#). Communications in Statistics - Theory and Methods 46:10582–10593.
- Lobry, J. R., M.-C. Bel-Venner, M. Bogdziewicz, A. Hacket-Pain, and S. Venner. 2023. [The CV is dead, long live the CV!](#) Methods in Ecology and Evolution 14:2780–2786.
- Pearson, K. 1897. [Mathematical contributions to the theory of evolution.—On a form of spurious correlation which may arise when indices are used in the measurement of organs](#). Proceedings of the Royal Society of London 60:489–498.
- Sapir, Y., and A. Shmida. 2002. [Species concepts and ecogeographical divergence of *Oncocyclus irises*](#). Israel Journal of Plant Sciences 50:119–127.
- Vallejo-Marín, M., M. E. Dorken, and S. C. H. Barrett. 2010. [The Ecological and Evolutionary Consequences of Clonality for Plant Mating](#). Annual Review of Ecology, Evolution, and Systematics 41:193–213.

References

- Wetzel, W. C., B. D. Inouye, P. G. Hahn, S. R. Whitehead, and N. Underwood. 2023. [Variability in Plant–Herbivore Interactions](#). Annual Review of Ecology, Evolution, and Systematics 54:451–474.
- Wilson, C. A., J. Padiernos, and Y. Sapir. 2016. [The royal irises \(*Iris* subg. *Iris* sect. *Oncocyclus*\): Plastid and low-copy nuclea data contribute to an understanding of their phylogenetic relationships](#). Taxon 65:35–46.
- Yenni, G. M., E. M. Christensen, E. K. Bledsoe, S. R. Supp, R. M. Diaz, E. P. White, and S. K. M. Ernest. 2019. [Developing a modern data workflow for regularly updated data](#). PLOS Biology 17:e3000125.

Part V.

Appendix 1: Forms & Checklists

22. Checklist - Onboarding Collaborators

22.1. Collaborator Checklist

New collaborators should:

- Review and sign the *Data Use Agreement*
- Review the *Authorship Guidelines* (Chapter 7)
- add required information to *Collaborator Contact Information* file
 - orcid id
 - link to github account
 - email
 - provide “expertise keywords”

22.2. Admin Checklist

HerbVar Admins then grant access to the following as appropriate:

- Slack
- HerbVar [Zotero Library](#)
- Github Access

23. HerbVar Forms

These links take you to the GitHub repository page that holds each document. GitHub will preview PDFs but not other file types. Either way, you can download the documents by clicking on the download button (downward pointing arrow) in the upper right of the



screen.

23.1. Data-Sharing & Usage Agreement

- [Data-Sharing and Usage Agreement](#) (.pdf format)

23.2. HerbVar Datasheets

Use the links to download datasheets for data collection and/or entry

- [HerbVar XLSX Datasheet for electronic data entry](#)
- [PRIMARY Datasheet \(PDF\), printable for use in field](#)
- [REPRO Datasheet \(PDF\), printable for use in field](#)
- [HERBIVORE Datasheet \(PDF\), printable for use in field](#)

23.3. HerbVar Field Guides

Use the links to download printable .pdf versions for use in the field.

- [Illustrated Guide to Percent Leaf Damage](#)
- [Mines & Galls Cheatsheet](#)

24. HerbVar Glossary

When writing papers using HerbVar data, authors should attempt to use a consistent set of definitions and terms, which can facilitate comparisons of patterns across studies. If one person’s “among-leaf variation” is the same as another person’s “within-plant variation”, this simple mis-match in terminology will hinder communication. The constellation of terms related to variance, variability, and variation can also be source of confusion. We have assembled a set of terms that might commonly be used in HerbVar manuscripts and grants. Many of the terms related to “distributional thinking” are defined in Wetzel et al. (2023) and we recommend referring to that paper for more detailed discussion.

24.1. About HerbVar data

Herbivory: Consumption of living plant tissues by animals (i.e. herbivores or omnivores). Many of the HerbVar data on herbivory are about leaf damage done by insects, rather than large mammals; we recommend that authors clarify this at some point in manuscripts.

Herbivore Damage: Plant tissue removed or directly injured by herbivore feeding. Some authors, especially those focused on agriculture, define damage to mean the growth, fitness, or yield consequences of injury, and reserve injury for the direct consumption effects.

Folivory: Damage to leaves by animals that consume leaves.

Frugivory: Damage to fruits by animals that consume fruits.

Reproductive damage: Damage on any reproductive structure, including flowers, fruits, and seeds.

24.2. Distributional thinking

Variability: The propensity for a process to produce different outcomes. Variability is a feature of underlying processes, whereas variation is a feature of an observed pattern (i.e., data). Well-known drivers of interaction variability in ecology include organismal plasticity, demographic stochasticity, and population dynamics; these factors and more combine to create an underlying probability distribution for a given interaction. When it is not possible to measure repeated outcomes, it may not be possible to measure variability directly.

Variation: The magnitude of differences among units in a system. Variability is a feature of underlying processes, whereas variation is a feature of an observed pattern (i.e., data).

Variance and standard deviation: A common way to quantify variation, the average squared deviation from the mean (or sum of squared deviations divided by (n-1) for samples

from populations). Note that the units of the variance are squared, so it is common to work with the square root of the variance, which is the standard deviation.

Coefficient of Variation (CV): The standard deviation divided by the mean. Many data-generating processes result in larger variances when means are larger (e.g., for Poisson processes the variance and mean are equal, by definition). Dividing the standard deviation by the mean helps to “correct” for this relationship, and results in a unitless number so that the CVs can be compared among any data, regardless of the original data units (leaves, area, height, etc.).

! Important consideration regarding the CV

The CV is popular, but is problematic when the mean is near 0 or negative (as can happen if data can take negative values as well as positive ones), or the variance is extremely large. A recently proposed alternative is bounded by 0 and 1 and is based on second-order distributional moments. It was proposed by Kvålseth (2017), thus is indicated by KCV, whereas the traditional CV is indicated here by PCV as it was first proposed by Pearson (1897). They are related by Lobry et al. (2023).

Gini Coefficient: The Gini Coefficient is a measure of inequality, rather than variance. It is bounded by 0 and 1, where 0 indicates a perfectly even distribution of a total amount among all individuals, and 1 indicates a perfectly unequal distribution of the total among individuals (all of the total belongs to one individual). Note that because the Gini is defined by a distribution of a total amount, it does not depend on the mean amount per individual.

Median Absolute Difference: The median of the absolute values of the differences between each value and the overall median. This is an alternative to the standard deviation that does not rely on squared values. It is less sensitive than the standard deviation to extreme values.

Mean Absolute Difference: The mean of the absolute values of the differences between each value and the overall mean. Like the median absolute difference, this metric is less sensitive than the standard deviation to large deviations.

Relative Mean Absolute Difference: The mean absolute difference divided by the arithmetic mean. Like the coefficient of variation (CV), this metric relativizes the mean absolute difference by the mean so that the dispersion of populations with different means can be compared. Like the CV, it is a unitless number. The relative mean absolute difference is equivalent to two times the Gini coefficient.

Skewness: A measure of the asymmetry of a distribution. A symmetric distribution, with the same shape of distribution tail in both directions, has a skew of zero. A positive skew indicates a longer tail toward higher values (the median is to the left of the mean). A negative skew indicates a longer tail to the left. In R, the ‘sn’ package can be useful for working with ‘skew-Normal’ distributions, extensions of Normal (Gaussian) distributions.

Kurtosis: A measure of the thickness (“fatness”) of a distribution’s “tails”. When a distribution’s tails are thicker, data are more likely to have a high variance and contain larger outliers.

24.3. Terms specific to the HerbVar data

A strength of the HerbVar dataset is that it contains data for multiple hierarchical scales of organization, from leaves on individual plants, up through populations and species. Patterns in the HerbVar data can be analyzed at multiple scales. Within a manuscript, we recommend people be clear about the scale(s) of analysis, and consistent about which term are used.

Among-leaf variation: This is the smallest scale of HerbVar data, the leaves on a single plant individual. This variation can be quantified using metrics such as the Gini coefficient, the variance, the standard deviation, or a CV. At this scale all of the damage is to a single individual, so it has also been called “**within-plant variation**”.

Among-plant variation: Each plant has a mean amount of herbivory, which can vary among plants within a population (for most HerbVar data, a population contains 60 individuals). This scale might also be called “**within-population variation**”.

Among-population variation: For some plant species, participants have contributed data from different populations. For focal species, the HerbVar data include many populations from multiple continents. Each population has a mean amount of herbivory, also known as “within-species” variation. Comparisons among populations are facilitated by having the same sample size for each population.

Among-species variation: For studies looking across species within a family, or at any larger phylogenetic context, one could compare species-level mean amounts of herbivory. This idea can be transferred to higher phylogenetic levels by looking at **among-family variation**, or to environmental scales of organization by looking at **among-habitat variation** or **among-biome variation**.

Part VI.

Appendix 2: HerbVar Admin

25. Potential Collaborators

Potential collaborators typically email either Will or the HerbVar Admin gmail address. If they contact Will, he replies thanking them for their interest and forwards the email to you for follow-up. **These are the steps for handling new expressions of interest in collaborating:**

1. Reply with the first email template (of 2) for prospective new members. Be sure to cc Will.
2. They respond and say:
 - a. **they're not interested/have issues with the expectations.** Send them over to Will and stay on top of the email chain between them so you can know how he handled it. *This hasn't happened, but in theory it could.*
 - b. **They say that sounds fine and they're still interested**
 - i. Add them to the Collaborator Contact Information file
 - ii. Add them as a "Contributor" to the HerbVar Shared Drive
 - iii. Respond with the second email template (of 2)

It is important you send the email after doing the steps i and ii because the email template includes links that assumes (1) you have entered preliminary information into the collaborator contact info file and (2) they have access to everything in the HerbVar Shared Drive. Note also that the second email template contains an onboarding document that you may need to update going forward as onboarding needs evolve.

26. Editing the Manual

26.1. Overview

This guide is a [Quarto Book](#) hosted on the [HerbVar Network's Github site](#). Any HerbVar member can edit the manual, add new sections, or make suggestions for improvement either via [pull request](#), [posting an issue](#) on the HerbVar Manual's repository, or editing the manual directly.

26.2. How to Edit the Manual

26.2.1. Getting Started

Quarto is the next-generation of R Markdown. Like R Markdown it allows for rendering files in multiple formats (.pdf,.docx., .html) and it can be used to make websites, reports, and presentations, but it has the advantage of being able to run embedded code chunks in multiple languages (e.g., R, Python) and allowing users to execute code (i.e., for demonstrations) in the rendered quarto file. Users of Markdown / R Markdown will recognize the structure and format of quarto files (.qmd), and the content of R Markdown files can be executed in .qmd files.



Tip

A tutorial on getting started with Quarto and RStudio can be found [here](#). Posit (*née* ‘R Studio’) has also prepared an excellent [Quarto Cheatsheet](#) that will prove very helpful.

To edit the manual you will need a Github account and RStudio. The process for editing the Manual is:

1. Install the `quarto` package
2. Fork the Herbvar Manual's Repository and clone it into your personal Github
3. Edit files (or create a new ones)
4. Commit and push the changes
5. Submit a pull request with your suggested edits

Step 1. Open R Studio and install the `quarto` package using either the ‘install’ button on Rstudio or by executing the following command.

Step 2. Fork the repository for the HerbVar Manual [[repo link](#)] from the HerbVar Organization Github into your personal Github account (tutorials [here](#) and [here](#)).

Listing 26.1 from the R Studio Command Line

```
install.packages("quarto")
```

Step 3: Edit files (or create a new ones). You can format your text using markdown syntax (see this [cheatsheet](#)), but if you would prefer to edit text and simply leave formatting notes (e.g., “bold this”, species names in italics”) that’s fine too.

Step 4: Commit and push the changes using RStudio (or the command line if you feel the need to flex).

Step 5: Submit a pull request so the Manual’s ‘Administrators’ can review and incorporate your suggested edits. (tutorials: how to submit a pull request from a forked repo [here](#), general introduction [here](#) and [here](#).)

26.3. Publishing the Manual (Admins Only)

The admins of the repo can publish the book in HTML (to a Github pages site) and as a pdf (on the repo). There are three ways for a Admin to publish Quarto documents to GitHub Pages(see [here](#)):

1. Render sites on their local machine to the docs directory, check the rendered site into GitHub, and then configure the GitHub repo to publish from the docs directory.
2. Render on their local machine and then use the `quarto publish` command to publish content rendered on the local machine.
3. Use a GitHub Action to automatically render files (a single Quarto document or a Quarto project) and publish the resulting content whenever a source code change (e.g., any edit of the manual) is pushed to the repository.

We are currently using Github Actions to automatically render and publish any time a change to the manual is committed and pushed to Github. However, if there is ever an issue with gh-action, or if you want to try the manual way of rendering/publishing, you can do the following: (1) Use the `quarto render` command from the Terminal to render all output formats (the output of your book will be written to the `_book` sub-directory of your book project), then (2) use the `quarto publish gh-pages` command to publish the locally rendered content online (more detailed instructions can be found [here](#)).

Rendering the manual:

Listing 26.2 from the Terminal

```
quarto render
```

Note that if you pass no arguments to `quarto render` as above, all formats will be rendered. You can instead render individual formats via the `-to` argument:

26. Editing the Manual

Listing 26.3 from the Terminal

```
quarto render          # render all formats  
quarto render --to pdf # render PDF format only
```

Publishing the manual:

Listing 26.4 from the Terminal

```
quarto publish gh-pages
```

27. Editing the Website

27.1. Github Access

1. The [HerbVar website](#) has been created in R. Ask to be added as a collaborator on [this repository](#).
2. Fork the [website's repository](#) to your computer. Nick Lyon initially forked the repository so that pushes would be preserved as “pull requests” and could be reviewed by Will before actually changing the website on the internet, but this may be unnecessary depending on your comfort with this type of coding.

27.2. Making Edits

Make whatever changes are asked for or required. Each page of the website is saved as a separate .Rmd file and file names mostly correspond to website tab names so it should be relatively easy to identify which script(s) needs to be changed and implement those edits. The existing scripts also include plenty of examples of heading formatting, font changes, and hyperlinks so use the existing pages to teach yourself how to do things you don’t already know how to do.

27.3. Rebuilding the Website

1. Once you’ve made the edits, go to the “Build” tab on R Studio and click “Build Website”. This will take several minutes to process (there will be a running list of code as it processes through each .Rmd file) so feel free to grab a cup of coffee as this processes.
2. Once it completes, it will create a new tab and will pop up the new website in your browser **but you are not yet done!**
3. Once that is done, in the Git tab of R Studio, select all modified files (not just the scripts!) and commit/push them all.
4. Building the website may affect a lot of files in the “libs” folder deep in your system (you can tell how savvy I am about this, huh?) and these changes must also be included in the push for the website to successfully update.
5. Once you’ve pushed these changes (and if you’re working in a fork, Will has accepted your pull request) the website on the internet should update within 10-15 minutes so double check your work after roughly that amount of time has passed.

28. Data Portal

The data portal [link](#) is the preferred method for data submission for (at least phase 2). It is written in R Shiny and is built for the phase 2 template Excel file but will work (with some warnings) for the phase 1 Excel template. This is what you need to know to change and/or troubleshoot the app.

28.1. Your Job After Someone Submits Data via the Portal

The data portal puts submitted data in the “App Uploads - Phase 2” folder. The portal would be self-sufficient but I have added a step to require human involvement that I’ll describe here.

1. You need to move all files from that folder to the “Phase II Raw Data” folder
2. All phase 2 wrangling scripts will download raw data from that latter folder
 - a. I’ve set up all of the wrangling scripts to download raw data in an `if() else{}` framework that will print a message reminding you to move the data out of the app upload folder if you ever forget to/don’t see that new data have been uploaded
3. That’s it! The data wrangling scripts will work without issue now that you’ve moved the files to the correct folder

28.2. Updating the Portal

It may become necessary to edit the portal, especially if a user emails you indicating they had a problem and it seems like that problem is inside of the app rather than (not to be mean) user error.

1. All of the portal code is in the “Data-Portal” GitHub repository
 - a. The script from which the portal is created is called “app.R” and is the only file in the folder “Data Portal Actual”
 - b. You will also need the “deployment-faq.R” script in the “Support Scripts” folder in order to deploy the app after you have made/tested any changes to the portal’s code. i. If it is of interest, the “Support Scripts” folder also includes my (Nick’s) incremental forays into the world of Shiny so you can see the first through eighth versions of the portal before getting to a version that was deployed.

2. Before changing the portal I strongly recommend asking the user who pointed out the issue for a screenshot of how they've filled the app out immediately before the error
 - a. The error is almost always (or at least has usually been) something to do with how the user filled out the app or attached their data. If that is the case, you may need only point that out to them (in a polite way) and go about your day
 - b. Also, the only time the app can break is when they click the “Submit Data” button. Prior to that, the app is not actually trying to do anything, so any app-breaking user error will not be apparent to them until they click that button
 - c. HOWEVER, some users who experience an issue actually create a larger error that will prevent them from uploading their data even after you point the app key issue out to them. To handle such cases, see the next subsection. The importance of this is also noted in part d of the next bullet 3. To change the data portal do the following:
 1. First, modify the app. R script as desired. Note that every Shiny App consists of three components: (1) the user interface, (2) the server that includes all the internal mechanisms for the portal, and (3) a ShinyApp() call that combines the UI and server.
 - i. If the app is not working, it will likely be in the server component
 - ii. If the app doesn't look right but does function appropriately, modify the UI
 - iii. If the app does not collect some information that it should, you will need to change both the UI and server
 - iv. The ShinyApp call at the end never needs to be modified so don't worry about that bit
 2. Second, test the app on your computer by running the app
 - i. In R Studio, the top right of the R script panel containing a ShinyApp has a “Run App” button to the right of a green ‘play’ button
 - ii. Pushing this button will create a local version of the portal that functions as the app will but does not deploy to the internet (yet).
 - iii. I recommend submitting a test data file (see the folder of the same name for pre-built phase 2 data that you can use) from start to finish to ensure that everything works as desired.
 3. Third, once you are satisfied with your changes, you can deploy the app to replace the old publicly-available version on the internet!
 - i. In the “deployment-faq.R” script, you will load the “rsconnect” library (line 12) and then use it to redeploy the app (line 18)
 - ii. Running the deployApp function will prompt you in the console to type a “Y” if you're sure that you want to re-deploy the app

- iii. After you type “Y” and hit return in the console, it will build your new portal, terminate the old one, replace the old with the new, and then activate the new one for all users
 - iv. You’ll know this is done when R automatically kicks you to a new tab in your web browser with the new portal open
3. Fourth, and this is crucial, if your changes to the app were because a user was having issues, you need to delete any files they successfully submitted
- i. See the next sub-section for information on how to do this/why it needs to be done
4. Finally, notify the user that initially contacted you letting them know that you have resolved the issue on your end, thanking them for bringing it to your attention, and inviting them to reach out again if it still isn’t working for them

28.3. Deleting Old Data

The data portal will fail if it tries to create two files of the same name.

- 1. The amount of information used to create the file name means that it is incredibly unlikely that two different users could accidentally create the same file name
- 2. BUT, as mentioned in the “Updating the Portal” section, it is entirely possible (and has happened previously) for the same user to try to submit data more than once and inadvertently create two files of the same name
 - a. This occurs when the following happens:
 - b. First, the user tries to submit data using the portal but something goes wrong so their data aren’t actually submitted (but a blank GoogleSheet of the user-supplied name is created)
 - ii. Second, the user tries to re-submit data (possibly after you fix the issue in the portal and notify them) but the blank document they unknowingly created earlier now causes a different error (i. e. , that there are now two files of the same name)
 - iii. Unfortunately, because Shiny Apps are noninteractive (see the “Service Account FAQ” section) the user will never be provided with an informative error (neither will you) so you’ll need to diagnose this as part of your ‘fixing the app’ process
- 3. To resolve this, I (Nick) have created a second Shiny App that is not deployed
 - a. To be clear, it should never be deployed to prevent its accidental (mis)use by general HerbVar members
- 4. Justification and location of the second app
 - a. The second app is in the “Data-Portal-Maintenance” GitHub repository

- b. The “Service Account FAQ” section below gives more context but in brief: the ‘app key’ file that the data portal makes users attach is actually activating a sort of Google robot with the authority to create Google Sheets and move them i. This is necessary because an online portal cannot send an authorization request to each user in the way that R/R Studio does when such code is run on a local computer (again, see the “Service Account FAQ” section for more details)
 - c. This ‘robot’ then is the true owner of all data files submitted through the app
 - d. The portal cannot submit data to a Shared Drive (due to issues with the R packages that connect R and Google that are outside of our control) so this is an unavoidable state
 - e. So, if a user accidentally creates a flawed data object of the same name as their real data they will be unable to submit their real data until the flawed one is deleted
 - f. HOWEVER, because the ‘robot’ owns those files, you cannot actually delete any of its files (when you “delete” a file you don’t own you actually just remove yourself as a collaborator with no effect on the original file)
 - g. Here is where we get to the need of a second app
 - h. The robot’s GoogleDrive cannot be accessed via a Graphical User Interface in the way that you would access any other Google Drive
 - ii. So, to truly delete these files so a user can re-submit their data successfully, you will need to use this second app
 - iii. If you fail to delete the bad data, the user will never be able to successfully submit data of the same name 1. In theory, you could ask them to re-name their file in some slightly different way (i. e. , by changing their site name), but that would still have this flawed data floating in the ether which is not desirable
5. Tutorial of the second app
- a. To reiterate, this Shiny app should never be deployed.
 - b. You will see why, but for the moment, take my word on it that deploying this app has a non-zero potential of permanently deleting data files you actually want
 - ii. By keeping the script in GitHub and locking view access to only Will & the Data Scientist, we preserve its utility without opening Pandora’s box of deploying it and possibly having an HerbVar member use it improperly
 - b. The second app is fully contained in the “check-service-acct-files.R” script (the only script in this project)
 - c. Open that script and click the “Run App” button in the top right of the R script pane of R Studio
 - d. As with updating the data submission portal, this will create a new tab in your web browser that contains a fully functional (but not available on the internet) version of the app

- e. The app is divided into three columns that you will proceed through from left to right
 - f. First, download and attach the key for the service account that owns the files you want to look through (column 1)
 - g. For now we only have one service account for phase
6. I recommend creating a new service account for each subsequent phase to evade data storage limits and partition sources of error in a clean, behind-the-scenes sort of way
- ii. See below for information on creating Service Accounts
- f. Second, click the “Authorize” button to notify the app that it should attach the app key (column 1)
 - g. This may take a few seconds but should generate a full list of all files owned by the robot (i. e. , owned by the service account) g. Third, after looking at the list of files, click the “Extract File Names” button (column 2)
 - h. This just populates the third column so don’t worry about the violence implied by the verb ‘extract’
 - i. Fourth, scroll through the drop down list (column 3) and select the file you want to delete (could be a test data file or the product of a specific user’s failed attempt to upload their data)
 - j. Fifth, above the dropdown menu, check the “Yes” option beneath “I am ready to delete a file”
 - k. I recommend doing this after selecting a file to further mitigate the risk of deleting the wrong file j. Sixth, click the “Delete Selected File” button i. Because you attached the service account key in column 1, you are viewing and interacting with the robot’s files as the robot (rather than as yourself)
 - ii. This gives you access to actually delete files rather than just—as mentioned before—removing yourself from seeing the file
 - k. Seventh, once a dialogue has popped up below the “Delete Selected File” button confirming the file has been deleted, click the “Update List of Drive Contents” button
 - l. This will update the dropdown menu with the new file list now that the file you marked for deletion has been erased l. Finally, scroll through the dropdown menu (or look at the list of files in column 2) to ensure that all problem files have been deleted
 - 6. After you’ve gone through that process to delete the flawed file, you can notify the user that it is safe for them to resubmit their data
 - m. This is all likely too much information for the user though so I suggest that you just tell them you have fixed the data portal and leave it at that
7. Also, I have written the app to work with any service account key that owns Google files so unless the structure of future phases’ data portals changes massively, this app should be sufficient for all issues involving service account-owned files in the future

28.4. Service Account FAQ Background Information

The data submission portal accepts uploaded data locally and then (1) creates a Google Sheet version of the data and (2) moves that sheet into the designated folder in the HerbVar Admin Drive. However, the Shiny app is “non-interactive” (see *gargle*’s vignette) which means that a user cannot input a gmail or access token to tell Google Drive/Sheets who is creating/moving files. A “service account” is necessary to get around this.

A service account is essentially a robot that we pre-approve to (1) create google sheets, (2) move files, and (3) have access to the folder(s) we want those sheets made in/moved to. Side note: see the list of people with access to the folder the Shiny portal saves files to and you’ll see the service account I created in that list.

To create/manage a service account you need to use “Google Cloud Platform” as described below:

28.5. Tutorial

1. Sign into the `herbvar@gmail.com` Google Account
2. Visit the Google Cloud Platform
 - a. If there is a pale red/pink error saying you don’t have sufficient permissions to view the page, select the `herbvar@gmail.com` account from the drop down in the top right of the screen
 - b. The page should then re-load to the dashboard
3. Don’t get overwhelmed by the level of detail on this page!
4. In the left sidebar, click “APIs & Services” and within that menu click “Credentials”
5. Click the service account name in the “Service Accounts” list at the bottom of the screen
6. Keys can be managed in the “Keys” tab
7. In the event of a security breach (not sure what that would look like but still good to have the contingency), delete the existing keys and create a new one
8. Download that key and replace the one HerbVar members have access to with the new key file.
9. If creating a new key or service account prompts you to add permissions to the account be sure that it includes BOTH the GoogleSheets API AND the GoogleDrive API
 - a. Both are needed because the data portal uses the service account key to both create a GoogleSheet (using the eponymous API) and move that GoogleSheet (using the GoogleDrive API)

29. Google Drive

This project has a lot of files coming and going so this is a brief description of all of those folders.

29.1. Phase I Data Wrangling

- HerbVar Phase I Data
- All Uploads
- All of the phase 1 raw data (and we've since moved to phase 2 so there should not be any more new data)
- Phase I
- Herbivore Data
- herbivoreData (from the eponymous Excel sheet) raw, tidied at plant-level (one row per plant) and tidied at survey level (one row per survey)
- Raw is the herbivore columns sliced off of the plantData sheet as part of the main wrangling script
- Phase I - Primary Productivity
- Primary productivity metadata to go along with phase 1 data
- Phase I - Reproductive Data
- Raw reproductive data columns sliced off of the plantData sheet as part of the main wrangling script
- Phase I - Richness Data
- Extracted native/invasive species richness from Ellis et al. 2012
- Phase I - siteData - Tidied version of siteData (from eponymous Excel sheet)
- Phase I - Survey Indices - Attempt (later abandoned) to split off just the site and plant-identifying information without any of the “actual” data. This would allow wide sharing of location information to enable all interested HerbVar members to harvest publicly-available metadata
- Phase I Data - Wrangled
- The “abiotic” (i.e., climatic) data for each site’s lat/long coordinates
- The tidied plant-level data (one row per plant) for all phase 1 data

29. Google Drive

- The tidied survey-level data (one row per survey) for all phase 1 data
- HerbVar Foliage Index.xlsx - Index describing whether each species (from each PI) is deciduous, evergreen, or annual (this information was integrated into the phase 2 data submission portal so no equivalent Google Sheet will exist for phase 2)

29.2. Phase II Data Wrangling

- _Data Submitted Via Email - I need to put it through the App - This is a clearinghouse for all raw phase 2 data that users send via email rather than using the submission portal. While discouraged, we don't want to completely block data in such instances. So, once you receive data via email, drop it into this folder until you have the time to run it through the app (the handful of times this has occurred the data went through the portal fine, users just got frustrated from unrelated things)
- App Uploads - Phase 2 - Raw data submitted via the portal arrive here

29.3. Phase II Completed Surveys Versions

- All phase 2 wrangling scripts copy the completed surveys file with a time stamp to this folder for posterity. I can think of no direct utility of these backups but it doesn't hurt to save them -

29.4. Phase II Metadata

- Rather than have separate folders for each metadata type (as was the case in phase 1) I have created this folder to contain them. Only abiotic (i.e., climatic) data have been retrieved so far but all should be placed here to keep the Drive folder hierarchy clean

29.5. Phase II Raw Data

- All data submitted through the submission portal should be manually moved from the "App Uploads - Phase 2" folder to here. The wrangling scripts will prompt you to do this if you do not before running them.

29.6. Phase II Wrangled Data

- Each sheet of the Excel file has its own version of the above folder where the ellipses (...) is replaced by that sheet's name. Where applicable (e.g., plantData, reproData, herbivoreData) there are survey-level (i.e., one row per survey) versions of the data

29.7. Miscellaneous Other Files

Note: this heading is not a folder name but refers to the random other files in the Drive.

- This manual is unfiled in the Drive!
- The Data Management Plan (DMP) in graphical form
- Tutorials for one-off tasks you may need to explain to others

30. Wrangling Repository

The [Wrangling Repository](#) contains scripts for data wrangling for all phases of the project. It takes in raw data and outputs analysis/visualization-ready .csv files.

It will be the primary home for this Research/Admin Position (or at least it was for me) so it may help to give you a brief explanation of each of the main scripts. Here's the link.

30.1. Misc. Non-Manuscript Subset Scripts

So far, this only includes the script to separate out PlantPopNet members' data to make sharing that with PPN leadership (upon request) simpler

30.2. Phase 1 Scripts

- `phase 1 abiotic wrangling.R`: Wrangles WORLDCLIM climatic data for phase 1 surveys.
- `phase 1 herbivoreData wrangling.R`: Wrangles any information to do with herbivores from phase 1.
- `phase 1 plant richness wrangling.R`: Extracts interpolated native/invasive species richness information from Ellis et al. 2012 shapefiles.
- `phase 1 primary productivity.R`: Extracts primary productivity data from satellite data for phase 1 surveys.
- `phase 1 shareable index.R`: Creates files of only location information for phase 1 sites to enable other HerbVar collaborators to harvest metadata without sharing “actual” data.
- `phase 1 siteData wrangling.R`: Wrangles information from siteData sheet of template Excel file.
- `phase 1 soil data wrangling.R`: Placeholder describing where soil data may someday be acquired. For now, the relevant R package does not work (though their team is aware of and working on this issue).
- `phase 1 survey-lvl summarizing.R`: Summarizes the tidy plant-level (one row per plant) phase 1 data to survey-level (one row per survey).
- `phase 1 wrangling.R`: Takes all the separate phase 1 raw data files combines and wrangles them to plant-level (one row per plant).

30.3. Phase 2 Scripts

- `phase 2 densityData wrangling.R`: Wrangles eponymous sheet from template Excel file.
- `phase 2 herbivoreData wrangling.R`: Wrangles eponymous sheet from template Excel file (at both plant-level and survey-level).
- `phase 2 metadataabiotic.R`: Extracts WORLDCLIM climatic data from phase 2 site locations (requires tidy file from siteData wrangling script).
- `phase 2 newColumns wrangling.R`: Wrangles eponymous sheet from template Excel file.
- `phase 2 notes wrangling.R`: Wrangles eponymous sheet from template Excel file.
- `phase 2 plantData survey-lvl summarizing.R`: Summarizes tidy plantData to survey-level (i.e., one row per survey).
- `phase 2 plantData wrangling.R`: Wrangles eponymous sheet from template Excel file (at ONLY plant-level).
- `phase 2 reproData wrangling.R`: Wrangles eponymous sheet from template Excel file at plant-level only (survey level absent because insufficient raw data at this point).
- `phase 2 siteData wrangling.R`: Wrangles eponymous sheet from template Excel file.

30.4. Script Archive

All “actual” scripts (i.e., those used in day-to-day wrangling) should have a consistent aesthetic and comment structure (as well as being primarily tidyverse-based). When others contribute code, duplicate the file and edit one version to match internal standards. The second version goes here to be preserved in its original form as a back-up

30.5. Singleton Tasks

Any scripts written to accomplish a ‘one-off’ task I thought unlikely to be repeated regularly are placed here. Some of them may include operations that could be useful in other contexts though!

30.6. Manuscript Subsetting Scripts

Each script is dedicated for a single HerbVar manuscript and does the subsetting and/or column selection necessary to create a tidy data file of only what authors request to test their hypotheses