HerbVar Project Manual & Field Protocols

The Herbvar Steering Committee

2024-05-03

Table of contents

Preamble **3**

1 What is HerbVar? **4**
1.1 Motivation 4
1.2 HerbVar Goals 4

I New Collaborators: Start Here **6**

2 First Steps **7**

3 Contact Information **8**

II Research Workflow **9**

4 Workflow Overview **10**
4.1 Collecting new HerbVar Data 10
4.2 New analysis with Herbvar Datasets 10

5 Data Collection **11**
5.1 Overview 11
5.2 Selecting species and sites to survey 11
5.3 Primary Protocol 11
5.4 Documents to help with herbivory estimation 12
5.5 Alternative survey protocols, for other plant types 12
5.6 Insects 12
5.7 Entering and Correcting Data 12

6 Uploading Data **13**

7 Data Analysis **14**

8 Preparing HerbVar Manuscripts **15**
8.1 Reproducible Manuscripts 15
8.2 Prior to Submission 15
8.3 Following Submission 15

8.4 Upon Acceptance 15

III Protocols **17**

9 Species & Site Selection (Phase 2) **18**

10 MOST RECENT UPDATE: **19**
10.1 Overview 19
10.1.1 Species Objective 1: Focal species across gradients 19
10.1.2 Species Objective 2: Novel species & contexts within focal families . . . 19
10.1.3 Species Objective 3: Damage to Reproductive Tissues 20
10.1.4 Site Selection Guidelines 20

11 Primary Protocol **21**
11.0.1 Overview 21
11.0.2 Note on Alternate Protocols 21
11.1 Ideal Focal Species Abundance 22
11.2 Before the Field 22
11.3 Primary Protocol 22
11.4 After the Field 25
11.5 Methods Notes 25
11.6 Guidelines for Picking Plant Species 26
11.7 Delineating a Site 26
11.8 When to Sample 27
11.9 Common Garden Data 27

12 Reproductive Damage **28**

13 Damage Estimation **31**
13.1 Overview 31
13.2 Objectives 32
13.2.1 1. Estimating Plant Size (and Determining What Tissue Counts) 32
13.2.2 2. Counting Number of Total and Damaged Leaves (up to 60) 33
13.2.3 3. Estimating Percent Damage on 10 Randomly Chosen Leaves 35
13.2.4 4. Estimating Percent Damage Across the Whole Plant 39

14 Low Density/Abundance Plants **41**
14.1 Overview 41
14.2 Protocols 41
14.2.1 Option 1 – Comprehensive Patch Census 41
14.2.2 Option 2 – Walking Transect 43
14.2.3 Option 3 – Comprehensive Census of Subset 43

15 Cacti & Succulents **45**
15.1 Overview . . . 45
15.2 Sampling Issues within Architectural Categories . . . 45
15.3 Expected Types of Herbivory . . . 46
15.4 Other Notes About Cacti . . . 47
15.5 Protocol . . . 47
15.6 Pre-Census Tasks . . . 47
15.7 Census . . . 48

16 Trees **50**
16.1 Overview . . . 50
16.2 Objectives . . . 50
16.3 Protocol – Tree Seedlings & Saplings . . . 50
16.4 Protocol – Mature Trees . . . 51
16.5 Selecting Leaves for Herbivory Estimates . . . 52

17 Rhizomatous Geophytes **53**
17.1 Overview . . . 53
17.2 Objectives . . . 53
17.3 Background . . . 53
17.4 Protocol – Rhizomatous Geophytes . . . 54
17.4.1 Calculate a custom radius for circular quadrats . . . 54
17.4.2 Genet life stage: seedling, vegetative, reproductive . . . 54
17.4.3 Herbivore damage in one of 3 ways: . . . 54
17.4.4 References . . . 55

18 Herbivores, Mines, and Galls **56**
18.1 Overview . . . 56
18.2 Objectives . . . 56
18.3 Sampling insects beyond "core" herbivores. . . . 57
18.3.1 Mines & Galls Visual Guide . . . 58
18.3.2 Leaf Mines – Blotch Mines . . . 58
18.4 Insect Herbivore ID Visual Guide . . . 58

IV Admin Tasks **60**

19 Potential Collaborators **61**

20 Editing the Website **62**
20.1 Github Access . . . 62
20.2 Making Edits . . . 62
20.3 Rebuilding the Website . . . 62

21 The Data Portal **64**
21.1 Your Job After Someone Submits Data via the Portal . . . 64
21.2 Updating the Portal . . . 64
21.3 Deleting Old Data . . . 66
21.4 Service Account FAQ Background Information . . . 69
21.5 Tutorial . . . 70

22 Google Drive Structure **71**
22.1 Phase I Data Wrangling . . . 71
22.2 Phase II Data Wrangling . . . 72
22.3 Phase II Completed Surveys Versions . . . 72
22.4 Phase II Metadata . . . 72
22.5 Phase II Raw Data . . . 73
22.6 Phase II Wrangled Data - 73
22.7 Miscellaneous Other Files . . . 73

23 Wrangling Repository **74**
23.1 Misc. Non-Manuscript Subset Scripts . . . 74
23.2 Phase 1 Scripts . . . 74
23.3 Phase 2 Scripts . . . 75
23.4 Script Archive . . . 75
23.5 Singleton Tasks . . . 75
23.6 Manuscript Subsetting Scripts . . . 76

24 To-Do List for Manual **77**
24.1 Data Use Agreement . . . 77
24.2 Authorship Guidelines Conversation . . . 78
24.3 Herbivore Protocol Gray Area . . . 78

References **79**

V Appendix **80**

HerbVar Publications **81**

HerbVar Publications **82**

Preamble

This book is a manual for researchers involved 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).

This guide is a Quarto Book hosted on the HerbVar Network's Github site, so any team members 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. A tutorial on getting started with Quarto and RStudio can be found here.

---

1 What is HerbVar?

1.1 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 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 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.*** We are a global collaboration to quantify the distribution of herbivory for diverse plant species in multiple ecosystems across the world.

1.2 HerbVar Goals

The goals of HerbVar are (1) to assess if variability in herbivory is indeed a common feature of plant–herbivore interactions, and **(2)** to examine how the amount of variability and skew varies among different types of plant species, herbivore communities, and ecosystems. Quantifying general patterns in the distribution of herbivore damage within populations would be a major contribution to our fundamental understanding of herbivory. In addition, identifying the factors that correlate with variability in herbivory would provide the field with a new paradigm for describing plant–herbivore interactions and allow us to generate novel hypotheses about the ecology and evolution of plant–herbivore interactions. Among the factors under consideration are:

- Plant species identity,
- Plant functional traits (from literature),
- Plant ecology (e.g., rarity),
- Herbivore species,
- Herbivore functional groups,
- Ecosystem type,

- Latitude,
- …and many others (e.g., seasonality, precipitation).

Part I

New Collaborators: Start Here

2 First Steps

1. Add info to Collaborator Contact Information file

- make sure you have:
  - orcid id
  - github account

1. Get added as "Contributor" to the HerbVar Shared Drive (see if this is still best way)
2. get added to slack
3. get added to herbvar zotero group
4. request access to github
5. Review Authorship Guidelines
6. Sign Data Use Agreement

3 Contact Information

Communicating with HerbVar collaborators:

Want to email the Steering Committee? Here's their contact info in a format that easily copy/pastes into an email "To" field

Need to convert this to a list-serve

> Nora Underwood nunderwood@bio.fsu.edu, Brian Inouye binouye@bio.fsu.edu, Susan Whitehead swhitehead@vt.edu, Phil Hahn hahnp@ufl.edu, Lee Dyer nolaclimber@gmail.com, Emilio Bruna embruna@ufl.edu, Ivalu Cacho ivalu.cacho@gmail.com, Karen Abbott kca27@case.edu, Will Wetzel william.wetzel@montana.edu

Part II

Research Workflow

4 Workflow Overview

4.1 Collecting new HerbVar Data

1. Review and Select Protocols
2. Collect Data
3. Create a repository for cleaning the data using the `new_dataset_template`.
4. Upload the clean version of the data using the portal

4.2 New analysis with Herbvar Datasets

1. Identify Questions, Submit for Review to make sure no overlap
2. Create a repository for harvesting, organizing, and analyzing the data to be used in the analyses using the `new_analysis_and_paper_template`.
3. This template can also be used to write the manuscript in markfdown using a template such as `papaja` or one of the 'rticles' templates.

5 Data Collection

5.1 Overview

Chapter 1: This describes the larger strategic vision and project goals of the HerbVar Network. Below are links to the HerbVar sampling protocols.

5.2 Selecting species and sites to survey

Chapter 9: This describes our approach to select plant species and/or sites to survey. In brief, we suggest collaborators strive to survey either (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.

5.3 Primary Protocol

Chapter 11: is the primary protocol for most sites and species. We designed this to work for most plant species and sites. If it will not work for your species or site for whatever reason, then please consider one of our alternative protocols or get in touch with the HerbVar Planning Group.

Chapter 12: If your plants have reproductive tissues (flowers, fruits, seeds), please follow this protocol to quantify damage to these tissues.

Datasheet - Excel File. 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.

Datasheet - Printable PDFs. 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.

5.4 Documents to help with herbivory estimation

Chapter 13: This is a detailed walk through the process of estimating herbivore damage on leaves and whole plants, including tips for different types of leaves and damage.

Illustrated Guide to Percent Leaf Damage. This is a visual guide to what different levels of percent damage look like on a leaf. We suggest printing this out and taking it to the field with you to aid in estimation of percent herbivory. Currently, it has leaves of two species, but more are on the way.

5.5 Alternative survey protocols, for other plant types

Chapter 14: If your plants are rare at your site—such that they occur at low densities or abundances—then please refer to this protocol.

Chapter 15: This document discusses issues related to quantifying herbivory on cacti and other succulents and outlines a protocol.

Chapter 16: This is a protocol for surveying mature trees for HerbVar. 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

Chapter 17: This is a protocol for rhizomatous species for which it is feasible to determine what constitutes a genet by identifying rhizomatous connections and for which genets are small enough that herbivory could feasibly be estimated on 30 genets and their nearest neighbors within a site.

5.6 Insects

Chapter 18: This document discusses *whether and how* to sample 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. The document also includes visual cheatsheets for pre-selected groups of insects and gall/mine counting.

5.7 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 7

6 Uploading Data

To upload data, please visit our data submission portal and be sure to use the template Excel file linked in Chapter 5

7 Data Analysis

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

8 Preparing HerbVar Manuscripts

Manuscript Preparation using the github repository / template

8.1 Reproducible Manuscripts

The same template for analyses can be used for creating a manuscript with Rmd

8.2 Prior to Submission

[ ] All HerbVar publications must include the following text in the Acknoweldgements:

"—- NSF Grant _____"

[ ] If the data set used has been archived at Dryad, please cite both the Dataset and the Paper as follows:

8.3 Following Submission

[ ] Add the MS to the Zotero Group Important for NSF reporting to know how many manuscripts are in review.

[ ] Post a Preprint (optional but encouraged)

8.4 Upon Acceptance

[ ] Update the record in the Zotero library

[ ] Archive the Data with Dryad

- only if the data are new, otherwise MS will indicate data source as Dryad

[ ] Archive the Analysis Repo on Zenodo

- instructions on freezing the data analysis code repo on zenodo

[ ] Archive the Manuscript Repo on Zenodo (optional)

- instructions on freezing the MS repo on zenodo

Part III

Protocols

9 Species & Site Selection (Phase 2)

10 MOST RECENT UPDATE:

10.1 Overview

Based on Phase 1 of data collection, we ask that you prioritize the following 3 sampling objectives in your site and species selections for Phase 2 of data collection:

10.1.1 Species 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.

10.1.2 Species 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.

10.1.3 Species Objective 3: Damage to Reproductive Tissues

Goal: Survey damage to any species' reproductive tissues (e.g., fruits, flowers, seeds, etc.).

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.

10.1.4 Site Selection Guidelines

Goal: 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. We can use any site anywhere around the world, but the most valuable sites will represent geographic regions, environmental conditions, habitats, or other ecological characteristics that we do not currently have in our database or that are currently 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.

Further Explanation: We realize that defining the 'edges' of a site can be subjective and not easy. Walk around and see if you see the density drop off to well below the mean density that is used to calculate radius size (see the Primary Protocol). This is usually quite simple; for example, 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.

11 Primary Protocol

Protocol for Quantifying Variability in Plant-Herbivore Interactions

Guidelines for Picking Plant Species Delineating a Site When to Sample Common Garden Data

11.0.1 Overview

Below, we provide a straight-forward and broadly applicable protocol to achieve project goals. 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. For more information on the broader motivations and goals of the project see here.

11.0.2 Note on Alternate Protocols

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. Alternative protocols can be found in the shared Drive in the "Protocols - Phase 2" folder. These include protocols for surveying the following: Low density/abundance populations Mature* trees * = surveys of immature trees (i.e., seedling/saplings) use the primary protocol Cacti and other succulents Reproductive damage Insect herbivores, galls, and mines Rhizomatous geophytes

If the primary protocol is not feasible for a species or site, then we suggest one of these alternative protocols. 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.

11.1 Ideal Focal Species Abundance

The primary protocol works best for sites with at least ~90 plant individuals, such that it makes sense to sample individuals randomly. If your site has fewer than ~90 individuals of your plant species, then please consider comprehensively censusing 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.

11.2 Before the Field

Please review HerbVar's Damage Estimation Training Guidelines

Note that information on precision of estimates and acceptable binning is contained in this document This document contains valuable information on how to estimate percent damage on various leaves Please spend some time training and testing yourself and anyone working with you in the field using 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 1 of the Damage Estimation Guidelines) The app has two stages, one in which you assess damage and are immediately told how close you are to correct and a second where you assess 50 leaves, and the results are given to you at the end. 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) Download the template datasheet for this protocol There are both digital (see siteData, densityData, and plantData sheets) and printable versions to facilitate standardized data entry If you have a question on this, please feel free to reach out to herbvar at gmail.com

11.3 Primary Protocol

Pick a plant species (see "Guidelines for Selecting Plant Species" below) Pick a site (see "Delineating a Site" below for advice) Pick a time to sample (see "When to Sample" below for advice) Determine a radius for your circular quadrats

If you are sampling one of the HerbVar focal species (Taraxacum officinale, Plantago major, or Plantago lanceolata): If you are using any other species, use the following process to determine a quadrat radius or use the table below:

Use a radius of 0.4 m for your quadrats Calculate your own quadrat radius by:

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 1) - Estimate mean density of plant / 1 m2 - Count the number of plants in 1 m2 at 10 random locations within the site - Calculate mean density (D) - Use D to calculate a circular quadrat radius (r) that would on average contain 4 plants r = 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).

Rather than calculating, you may also use this pre-calculated set of radii (Table 1) for non-focal species. Remember, for focal species, please use 0.4 m

Table 1. Pre-Calculated Quadrat Radii Number plants/m2 (Density D) Quadrat radius (r) D 0.1 3.6 meters 0.1 < D 0.25 2.9 m 0.25 < D 0.5 1.9 m 0.5 < D 1 1.35 m 1 < D 3 0.9 m 3 < D 6 0.55 m 6 < D 10 0.4 m 10 < D 20 0.3 m 20 < D 0.23 m

Lay a transect through the middle of the site (Fig. 1) Record GPS coordinates of origin, length (m), and compass direction (degrees) of transect (need to pick a coordinate system and precision) Select center points of circular quadrats (Fig. 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. For each quadrat: Locate a quadrat center point using transect and measuring tape or stick (Fig. 1) Count and record the number of focal plants within r meters of the center point (Fig. 1) See above for explanation of how to calculate r(or use values in Table 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 Record other quadrat-level data 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 Percent cover of all non-focal plant species (ignore focal species) These 2 percent covers could total more than 100% if they overlap If surveying understory plants, ignore forest canopy when estimating percent cover If the circular quadrat has 0 plants, record a zero and continue to the next quadrat If the circular quadrat has > 0 plants: Randomly choose 1 of the plants within the quadrat to survey A quicker 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. Be careful about over-representing large and/or isolated plants (which will be closer to more points relative to small plants in crowded patches). For the selected plant (1 per quadrat) record plant life 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) For the selected plant record plant size Use judgment to pick best measure for your species E.g., standing plant height (ground to tallest living part), stem length, foliage

diameter, stem diameter Perhaps most importantly: for the selected plant record herbivore damage in 3 ways (see Damage Estimation Training Guidelines) Note that "herbivore damage" includes damage caused by both vertebrate and invertebrate herbivores Presence/absence of leaf damage: Record both (A) the total number of leaves on the plant and (B) the number of leaves with >0.5% herbivory If the plant has 60 leaves total, please record the true numbers If the plant has >60 leaves total, randomly (arbitrarily) choose 60 and record those values. Please also make a note that you stopped at 60 (see template datasheet). Note that we are no longer including undamaged leaves in the following step, so these two data points are vital in understanding the proportion of the plant that is not damaged by herbivores. If plants have reproductive parts (flowers/fruits/seeds) that could have been damaged by herbivores, please see the Reproductive Damage Protocol. This is optional but encouraged. Estimated percent damage on 10 randomly (arbitrarily) chosen leaves with herbivory damage (> 0% herbivory) One estimate per leaf (for a total of 10 estimates) Please strive to sample in a way that selected leaves will be 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., LeafByte, 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. 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 If damage is estimated visually and leaves are visibly damaged but < 0.5% (i.e., damage > ~0.1% but 0.5%) record 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. Estimated percent damage across the whole plant, optionally also breaking apart damage by type or even species of herbivore if possible (e.g., sucking damage versus chewing damage, add columns as needed) E.g., If a plant has 4 equally sized leaves and 2 of those leaves are 50% eaten, then whole plant has 25% herbivory But take leaf size into account when leaves vary in size If this measure is not feasible to collect, measure 30 leaves instead of 10 in step 2 (see above) and leave this blank. Those 30 can then be used to calculate this value post hoc Record 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 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) 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) Data to record for the first nearest conspecific neighbor of selected plant: All the same data as focal plant except nothing for neighbor's neighbor Continue visiting the randomly selected points until 30 focal plants and 30 nearest neighbors have been surveyed

11.4 After the Field

Enter your field-collected data into the template Excel file Refer to the dataDictionary sheet if column meanings are unclear Use the data submission portal to upload your data The portal has numbered steps to assist the upload process 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

Fig. 1. A diagram of the sampling scheme described in the text. (1) Record plant density in 10 randomly located 1-m2 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.

11.5 Methods Notes

Modifications of this protocol may be necessary to adapt it to different systems (see "Note on Alternate Protocols" above). If the primary protocol won't work for your system, please first consult our alternative protocols (see protocol above and "Protocols - Phase 2" folder). 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. In Phase 1, collaborators reported that one survey (~60 plants) took between 0.5 and 2 person days (4-16 hours) using the methods above (after a species and site have already been selected). 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). 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). 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 a dedicated alternate protocol for surveying such species (see Rhizomatous Geophytes Protocol) Please see our Damage Estimation Training Guidelines for guidelines on how to

estimate herbivore damage. Here are two tips: 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). 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. 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.

11.6 Guidelines for Picking Plant Species

From the patterns found in the first phase of data collection we have developed the following objectives going forward: Surveys of the three focal species (Taraxacum officinale, Plantago lanceolata, and Plantago major), especially across broad environmental and/or geographic ranges 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. 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.

11.7 Delineating a Site

We realize that defining the 'edges' of a site can be subjective and not easy. We search for 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. 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.

11.8 When to Sample

This will depend on the natural history of the system. We will accept data sampled 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 (and sample when is feasible; some data is better than no data!). And repeat sampling is acceptable.

11.9 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.

12 Reproductive Damage

MOST RECENT UPDATE:

(NEEDS TO INSERT PHOTOS from https://docs.google.com/document/d/1Rz6wPDXpfEnv1M16gJ1a2D3DH
/edit)

Please send any comments or suggestions you have regarding the protocol to the authors or post as issues/pull requests on the repository (see the Manual Preamble).

Contents Protocol Data Recording Tips

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

Objectives: The goal is to measure the proportional damage to reproductive organs on each plant within the surveyed population. That is, for each individual plant we will record the number of damaged and undamaged reproductive organs. 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, although reproductive damage measurements are welcome from other plant populations from which no leaf damage measurements have been taken.

Protocol Select a species to survey We are hoping to get broad taxonomic and geographic coverage of damage to reproductive organs. 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: At least half of the individuals at your site should possess reproductive material. If most of the plants are in a vegetative stage, you probably won't be able to survey enough reproductive individuals to get a decent sample size. Ideally, >30 of the sampled plants will have reproductive damage data Each individual plant should produce enough flowers/fruits/seeds so that you can survey between 15-30 reproductive units per plant. These 'units' could be flowers, fruits, or seeds, whichever will give you enough things to count. E.g., if your plants have just a few fruits, try opening fruits and counting a random sample of seeds, which could get you higher numbers. If some plants have fewer units (~5-10 flowers/fruits/pods/seeds), that is okay, as long as most have at least 15. If your species typically has only one or a few flowers, we have provided a modified protocol below Most of the plants should be in a similar phenological 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 organ

type. Additionally, different phenological stages will likely be attacked by different insects If your plant does not meet these requirements, please skip measuring damage to reproductive organs. That's okay. Or get in touch if you have questions Use the Primary Protocol to establish a transect, pick/calculate a quadrat radius, and randomly select focal/nearest neighbor plants Identify the reproductive tissue you will be surveying E.g., flowers, fruits, pods, seeds, etc. For each plant then record (see Table 1): Phenology of the plant (i.e., budding, flowering, fruiting, etc.) Total number of units examined Herbivore damage per unit Either percent or count of injuries Pathogen damage per unit Unknown damage per unit I.e., any damage not attributable to either herbivores or pathogens Ideally at least 30 reproductive units will be examined per plant. For plants that have much more than 30 units, examine a haphazard subsample of 30 units per plant. These should be sampled from different parts of the plant to obtain a good representative sample of damage levels to the plant. Damage measurements will be highly variable among species, depending on the types of flowers or fruits produced as well as the type of damage that is most common in the population. Below we give some general guidelines. 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. For example, if your species has only one or a few flowers/fruit, you can estimate the damage as a percentage. Keep in mind that the 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.

Data Recording Tips Record the following data in the provided printable and digital datasheets. Start by surveying the type of damage that 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 into the seeds or fruits (looks like little black dots on the fruits). In many cases you will need to tear open the seed head/fruit to look for boring insects inside the seeds/fruit. Look for signs of chewing damage inside the fruit, such as destroyed seeds and insect frass. The best measurements of damage will depend on the type and extent of damage present. If the plant species experiences damage to multiple organs (e.g., petals, stamens, etc.), focus on the damage to the primary reproductive parts if it is not feasible to measure multiple organs. In the "reproUnit" column of the reproData sheet in the template Excel datasheet, please record what organs you are recording damage on (e.g., stamens, petals for flowers). If present and identifiable, record the number and identity of the florivore, frugivore, seed predator If your species has only one or a few flowers per plant and it's possible to record the percent damage to a flower or fruit, you can record these percent damages in the percR# columns. 15 such columns are provided but please add more if you are able to record the per-unit damage of more than 15 reproductive structures Indicate whether you are recording 'count' or 'percent' damage in the 'damageUnit' column.

Table 1. Example reproData information

Note 1: numReproHerb can be less than the sum of the counts of types of damage because a single reproductive unit can have multiple types of damage. Note 2: In the damageUnit

column, record ‘count’ or ‘percent’ depending on whether you are recording the number of damage reproductive units or the percent damage to a single unit

Examples of different types of damage to reproductive material

Abronia umbellata with chewing damage to two corolla (one is completely chewed and one is partially chewed). In this example, each floret would be scored for damage (2 damaged, 13 organs examined). Photo: Eric LoPresti. Abronia turbinata with damage and frass from a moth caterpillar (Neogrotella macdunnoughi). Note chewing damage to petals/corollas and caterpillars at base of florets. In this example, each of 30 florets would be scored for damage. Photo: Eric LoPresti.

Vicia americana seed pod. Notice boring holes in the upper-left seed and frass in the pod. In this example, each of 30 seeds would be scored for damage. Photo: Phil Hahn.

Monarda fistulosa seed head ripped open to reveal a weevil larvae. Notice the damage holes to the floral tubes. In this example, each flower head would be recorded as damaged (or undamaged) and ideally 30 flower heads would be assessed per plant. Often this species does not produce 30 flowers per plant, so smaller numbers would be acceptable. If most plants have fewer than 10 flowers, this would not be a good population to survey for reproductive damage. Photo: Phil Hahn. Lonicera fruit with chewing damage. In this example, each of 30 fruits would be scored for damage. Photo: Susan Whitehead.

Lonicera fruit with piercing damage. In this example, each of 30 fruits would be scored for damage. Photo: Susan Whitehead. Aquilegia shockleyi with chewing damage to fruits (from Heliothis phloxiphaga). In this example, each fruit could be scored for damage, or each fruit could be opened and seeds could be counted (infer missing, fully-consumed seeds from pod features). Photo: Eric LoPresti.

13 Damage Estimation

MOST RECENT UPDATE:

NEED TO INSERT PICTURES FROM https://docs.google.com/document/d/1Y90zfQBkluuhelDdb5WksW_8

1. Estimating Plant Size (and Determining What Tissue Counts)
2. Counting Number of Total and Damaged Leaves (up to 60) Subsampling Method 1 – Nose-Pointing Subsampling Method 2 – True Randomization Subsampling Method 3 – Arbitrary Sampling
3. Estimating Percent Damage on 10 Randomly Chosen Leaves
4. Estimating Percent Damage Across the Whole Plant

13.1 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.

Estimating plant size (and determining what tissue counts)

Counting number of leaves and number of damaged leaves (up to 60 max)

Estimating percent damage on 10 randomly chosen leaves

Estimating percent damage across the whole plant

** If you are just looking for quick tips on estimating percent damage, skip to #3 (page 5) **

Two related documents in the "Damage estimation training" folder in the Shared Drive are

A field guide to types of plant damage, which has photos of different types of damage

An illustrated guide to amounts of percent damage, which shows images of leaves with different percent damage to help you calibrate your visual estimates.

13.2 Objectives

First off, it is good to keep in mind the goals of these estimates. Ideally, we would like to know the total amount of plant material consumed by herbivores (e.g., grams of tissue eaten) and the total amount of plant material (e.g., grams remaining). Unfortunately, this is infeasible for most systems, so our goal is to approximate it. We will do this by estimating plant size and 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, then 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 in the "Alternative protocols" folder. 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 in the Succulents folder in the "Alternative protocols" folder.

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 in the "Alternative protocols" folder in the HerbVar Shared Drive for how to record this damage. The rest of this document focuses on damage to vegetative tissue.

13.2.1 1. 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 (aboveground unit) to be a unique "individual." Move leaf litter to look for aboveground 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 (like the brown leaf of the Lepidium plant on the right) (perhaps unless 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.

13.2.2 2. Counting Number of Total and Damaged Leaves (up to 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.

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.

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.

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:

13.2.2.1 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 (below). 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.

Is that creep trying to pick his nose with his thumb? No. This is the nose pointing method to acquire a random sample of plant parts. The creep/researcher (left) establishes a fixed point in his field of vision with an "L"-shaped hand position while turned away from the plant. He then turns to the plant, opens a single eye, and chooses the leaf that he is pointing to (right).

13.2.2.2 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 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!).

13.2.2.3 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.

13.2.2.4 Subsampling Method 4 – Make Your Own

Design (and make notes of!) your own subsampling scheme. Can you choose every seventh (or random-numberedth 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).

13.2.2.5 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 [left]), 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.

13.2.3 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.

Randomly choose 10 leaves:

If your plant has 10 or fewer leaves, then please examine them all; 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.

Estimate percent damage on each leaf:

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. 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, 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.

How to estimate damage

Record estimates at a high resolution. We usually record at a resolution of 2.5% (Table 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 1. Recommended resolution for recording percent herbivory

Percent Meaning 0% No herbivory 0.5% A trace amount of herbivory 1% ~1% herbivory 2.5% ~2.5% herbivory 5% ~5% herbivory 7.5% ~7.5% herbivory So forth Up to 100% (e.g., everything removed except base of leaf petiole)

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? 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…

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… Mentaly 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!

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.

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.

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.

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 If you have marginal damage on leaves with non-smooth margins: If you draw an entire margin a third of the way between the base of the margin teeth 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 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.

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.

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.

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

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.

How to make sure you're doing a good job

Be conscious that most people overestimate low levels of tissue damage (Johnson 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. Invest time in practicing, calibrating, and validating estimates. Especially do this before collecting data, and continue to calibrate and validate regularly through data collection.

Standardize among observers you work with or have a single observer for all estimates.

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.

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.

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.

Three steps in measuring damage with Image J

13.2.4 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 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. 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.

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

14 Low Density/Abundance Plants

Most Recent Update:

14.1 Overview

This protocol outlines three methods for surveying sites where the focal plant 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.

14.2 Protocols

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.

14.2.1 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.

Record spatial information via one of two methods:

1) **Option 1A - 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.
2) **Option 1B - 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.

    - 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.
    - 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).
    - After recording this information, follow the Primary Protocol as closely as possible

Other useful information

1. Record `popDiameter1` and `popDiameter2` as the approximate extents of your patch/census area
2. 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`).
3. 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).

4. 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!

14.2.2 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.

14.2.3 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 and see other notes for method #1 above.

1. **Option 3A - 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
    a. Start by randomly picking a transect starting point and direction
    b. 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)
    c. Keep going until you get at least 60 plants

2. **Option 3B: 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. The cons of this approach are that 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''.

    a. Explore outwards from your random starting point, surveying every plant you encounter until you get to at least 60 plants. *We do not recommend doing this unless your plants truly are all widely dispersed.*

15 Cacti & Succulents

MOST RECENT UPDATE:

Sampling Issues within Architectural Categories Expected Types of Herbivory Other Notes About Cacti Protocol Pre-Census Tasks Census

15.1 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.

15.2 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 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.

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. 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).

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.

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.

15.3 Expected Types of Herbivory

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

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.

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 englemanii as well that should be watched out for.

15.4 Other Notes About Cacti

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.

It seems likely that the newest, tenderest units (particularly in categories 4 and 5 cacti) are particularly likely to be attacked.

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).

15.5 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 (e.g., 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.

15.6 Pre-Census Tasks

Pick a species to census.

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.

Decide on a maximum number of pads per plant to census.

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: 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)

Practically, it's very hard to determine and quantify what is herbivory vs physical damage on older pads Physically, it can be hard and dangerous to access older pads on spiny plants!

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.

Ideally, investigate the major types of damage you may see, potentially making a cheat sheet of photos. See how long the protocol outlined below would take, then modify as necessary.

Please take detailed notes on any modifications made

15.7 Census

Record site characteristics (e.g., date, site, plant ID, etc.)

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.

See above discussion of architectural categories of cacti/succulents

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:

Pick transect and subtransect distances that will encompass your site and lay the transect through the site. Estimate the density of plants in the population.

Use the estimated plant density to calculate a quadrat radius to use for the survey.

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.

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: 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.

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.

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).

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.

Note presence of pathogens.

16 Trees

MOST RECENT UPDATE:

Protocol – Tree Seedlings & Saplings Protocol – Mature Trees Selecting Leaves for Herbivory Estimates

16.1 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.

16.2 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.

16.3 Protocol – Tree Seedlings & Saplings

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

16.4 Protocol – Mature Trees

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:

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.

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).

For trees that are at low density or low abundance, please consult our Low Density Protocol 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 See Primary Protocol and Damage Estimation Training Document for guidelines on quantifying percent herbivory per leaf

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.

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

16.5 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 right). 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

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 the Damage Estimation Training Document).

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.

17 Rhizomatous Geophytes

17.1 Overview

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, 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'?).

17.2 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.

17.3 Background

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) (terms following Harper 1977 and Herben and Klimešová 2020). The extent of clonal growth defines the spread of the genet, and is on a continuous scale of density (Vallejo-Marin et al. 2010). See Figure 1 for examples of two density levels of genets in irises.

17.4 Protocol – Rhizomatous Geophytes

1. 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.
2. Follow the Primary Protocol & Site Selection Protocol to pick a site, set up a transect, and randomly select focal quadrats' locations

17.4.1 Calculate a custom radius for circular quadrats

Estimate mean density of genets per square meter by counting the number of plants in 1 m2 at 10 random locations within the site

- 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.
- If a quadrat has >0 focal plants, randomly choose 1 of the genets to survey and record the following data:

17.4.2 Genet life stage: seedling, vegetative, reproductive

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

17.4.3 Herbivore damage in one of 3 ways:

1. Total number of leaf fans (ramets)

- For genets with >100 ramets, write "100" and make a note that your estimate was capped at 100 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.
- 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

Note that 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

2. If a quadrat has 0 focal plants, record a 0 and move to the next quadrat
3. Record the same data for the first nearest conspecific neighbor (of a different genet) that you recorded for the focal plant.
4. Continue visiting randomly select points until 30 focal genets and 30 nearest neighbor genets have been surveyed

Figure 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.

17.4.4 References

Harper, J. L. 1977. Population Biology of Plants. Academic Press, London.

Herben, T., and J. Klimešová. 2020. Evolution of clonal growth forms in angiosperms. New Phytologist 225:999-1010.

Sapir, Y., and A. Shmida. 2002. Species concepts and ecogeographical divergence of Oncocyclus irises. Israel Journal of Plant Sciences 50:S119-S127.

Vallejo-Marin, 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.

Wilson, C. A., J. Padiernos, and Y. Sapir. 2016. The royal irises (Iris subg. Iris sect. Oncocyclus): Plastid and low-copy nuclear data contribute to an understanding of their phylogenetic relationships. Taxon 65:35-46.

18 Herbivores, Mines, and Galls

MOST RECENT UPDATE:

TODO: NEED TO INSERT IMAGES FROM: https://docs.google.com/document/d/1l6ucJc6JpohcgPXBhg9e3/edit

Protocol Mines & Galls Visual Guide Insect Herbivore ID Visual Guide

18.1 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.

18.2 Objectives

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, if you are confident in insect ID (see below for specifics) please also collect insect herbivore data ## Protocol

Deciding whether to sample "core" herbivores. Please use the following questions to help you decide.

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

Grasshoppers/crickets/katydids (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" Auchenorrhynchans in the column provided in the template Excel file Non-Aphid Sternorrhynchans

This includes whiteflies, scale insects, and mealybugs

Are you confident that you can visually detect* the herbivores on the selected plant species (consider 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!

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!

18.3 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 herbivorous count 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

18.3.1 Mines & Galls Visual Guide

Leaf mines – Linear/Serpentine

Left/right = single mine, center = multiple mines

How do you count multiple 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 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.

18.3.2 Leaf Mines – Blotch Mines

Several examples of multiple blotch mines on single leaves

Galls – Leaf Galls

Galls – Stem/Branch Galls

18.4 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.

Grasshoppers / crickets / katydids (Orthoptera)

Grasshoppers (suborder: Caelifera); crickets/katydids (suborder: Ensifera)

May be confused with: mantids (left) or hoppers (right) Synapomorphies & Identifying Marks: Have swollen "knee" joints of hind legs (femoro-tibial joints), "chewing" mouthparts (mandibulate) and forelegs are never raptorial

Caterpillar-like (larval forms ONLY)

Lepidopteran caterpillars; sawfly larvae; herbivorous beetle larvae

May be confused with: syrphid larvae (left) or coccinellid larvae (right)

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.

Hoppers (Hemiptera: Auchenorrhyncha)

Leafhoppers, treehoppers, planthoppers, and cicadas

May be confused with: true bugs (i.e., Hemiptera: Heteroptera)

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

Aphids (Hemiptera: Aphididae)

May be confused with: whitefly

Synapomorphies & Identifying Marks: Have a pear-shaped body and may/may not have wings. Pair of tubes (cornicles) projecting from rear often present

Non-Aphid Sternorrhynchans (whiteflies, mealybugs, scale insects)

Whitefly (Aleyrodidae); mealybug (Pseudococcidae); scale insect (Coccoidea)

May be confused with: aphids

Synapomorphies & Identifying Marks: Relatively distant relatedness among these three taxa but each is relatively small (i.e., <5 mm) and distinctive. Mealybugs and scales are often found clustered on stems at high densities.

Part IV

Admin Tasks

19 Potential Collaborators

Potential collaborators typically get 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.

20 Editing the Website

20.1 Github Access

The HerbVar website has been created in R. Ask to be added as a collaborator on this repository.

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.

20.2 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.

20.3 Rebuilding the Website

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.

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**!

Once that is done, in the Git tab of R Studio, select all modified files (not just the scripts!) and commit/push them all.

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.

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.

21 The 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.

21.1 Your Job After Someone Submits Data via the Portal

The data portal puts submitted data in the "App Uploads - Phase 2" folder (link). 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 (link)
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

21.2 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 (link)
    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

21.3 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 (link)

    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

21.4 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:

21.5 Tutorial

1. Sign into the herbvar@gmail. com Google Account
2. Visit the Google Cloud Platform (link)
    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)

22 Google Drive Structure

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

22.1 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
- 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

22.2 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

22.3 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 -

22.4 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

22.5 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.

22.6 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

22.7 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

23 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.

23.1 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

23.2 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).

23.3 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 metadata`abiotic.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.

23.4 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

23.5 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 tough!

23.6 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

24 To-Do List for Manual

(as of 2/1/22)

1. Edit the "Google Drive" chapter
2. Convert "data portal troubleshooting" to a flowchart/checklist
3. Edit Protocols:

a. Insert Photos
b. link to pdf versions for downloading by users
c. edit / correct formatting

4. review and expand the workflow chapter
5. review and expand the data analysis chapter; include tutoriual for repo
6. review and expand the publications chapter; include tutoriual for repo
7. References/multiple .bib files
8. herbvar publications, herbvar presentations
9. add tutorial for editing the manual and publishing with actions https://quarto.org/docs/publishing/github-pages.html

---

Unfortunately I (Nick) took a new position while there were still some loose ends left hanging but I can list them here so they don't completely fall between the cracks -

24.1 Data Use Agreement

I wrote a data use contract for HerbVar members to sign upon joining the Network so that we can have them release rights to their data to us (and promise not to share the larger datafiles produced by the collaboration - The email conversation for getting the Planning Group's feedback and green light has the subject line "HerbVar Data Sharing Agreement Draft" - The relevant Google Drive folder is linked here and lives in the "HerbVar Management" Shared Drive -

24.2 Authorship Guidelines Conversation

The Planning Group was in the midst of deciding on revised authorship guidelines to catch some edge cases that fell outside of the earlier authorship policy. - You can catch up on this in the email thread with the subject “HerbVar Authorship Criteria Questions”

24.3 Herbivore Protocol Gray Area

The Herbivore protocol is written to prioritize taxonomic identification of insects. However, many researchers are also (and sometimes more) interested in functional identification of insects. Our current approach to the template Excel file allows people to add their own columns as they see fit but this makes later switching from functional to taxonomic (or vice versa) difficult. So, there is a draft email in the herbvar@gmail account to send when a panel of herbivore experts has been assembled to resolve this uncertainty

References

A .bib file of all HerbVar publications is available for download here.

Harper, John L et al. 1977. "Population Biology of Plants." *Population Biology of Plants.*

Herben, Tomáš, and Jitka Klimešová. 2020. "Evolution of Clonal Growth Forms in Angiosperms." *New Phytologist* 225 (2): 999–1010. https://doi.org/10.1111/nph.16188.

Pan, Vincent S., and William C. Wetzel. 2024. "Neutrality in Plant–Herbivore Interactions." *Proceedings of the Royal Society B: Biological Sciences* 291 (2017): 20232687. https://doi.org/10.1098/rspb.2023.2687.

SAPIR, YUVAL, and AVI SHMIDA. 2002. "Species Concepts and Ecogeographical Divergence of Oncocyclus Irises." *Israel Journal of Plant Sciences* 50 (January): 119–27. https://doi.org/10.1560/DJXH-QX0M-5P0H-DLMW.

The Herbivory Variability Network* †, M. L. Robinson, P. G. Hahn, B. D. Inouye, N. Underwood, S. R. Whitehead, K. C. Abbott, et al. 2023. "Plant Size, Latitude, and Phylogeny Explain Within-Population Variability in Herbivory." *Science* 382 (6671): 679–83. https://doi.org/10.1126/science.adh8830.

Vallejo-Marín, Mario, Marcel E. Dorken, and Spencer C. H. Barrett. 2010. "The Ecological and Evolutionary Consequences of Clonality for Plant Mating." *Annual Review of Ecology, Evolution, and Systematics* 41 (1): 193–213. https://doi.org/10.1146/annurev.ecolsys.110308.120258.

Wetzel, William C., Brian D. Inouye, Philip G. Hahn, Susan R. Whitehead, and Nora Underwood. 2023. "Variability in Plant–Herbivore Interactions." *Annual Review of Ecology, Evolution, and Systematics* 54 (1): 451–74. https://doi.org/10.1146/annurev-ecolsys-102221-045015.

Wilson, Carol A., Justin Padiernos, and Yuval 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 (1): 35–46. https://doi.org/10.12705/651.3.

Part V

Appendix

HerbVar Publications

A .bib file of all HerbVar publications is available for download here.

This will eventually have a list of all publications from the HerbVar Project

Harper, John L et al. 1977. "Population Biology of Plants." *Population Biology of Plants.*

Herben, Tomáš, and Jitka Klimešová. 2020. "Evolution of Clonal Growth Forms in Angiosperms." *New Phytologist* 225 (2): 999–1010. https://doi.org/10.1111/nph.16188.

Pan, Vincent S., and William C. Wetzel. 2024. "Neutrality in Plant–Herbivore Interactions." *Proceedings of the Royal Society B: Biological Sciences* 291 (2017): 20232687. https://doi.org/10.1098/rspb.2023.2687.

SAPIR, YUVAL, and AVI SHMIDA. 2002. "Species Concepts and Ecogeographical Divergence of Oncocyclus Irises." *Israel Journal of Plant Sciences* 50 (January): 119–27. https://doi.org/10.1560/DJXH-QX0M-5P0H-DLMW.

The Herbivory Variability Network* †, M. L. Robinson, P. G. Hahn, B. D. Inouye, N. Underwood, S. R. Whitehead, K. C. Abbott, et al. 2023. "Plant Size, Latitude, and Phylogeny Explain Within-Population Variability in Herbivory." *Science* 382 (6671): 679–83. https://doi.org/10.1126/science.adh8830.

Vallejo-Marín, Mario, Marcel E. Dorken, and Spencer C. H. Barrett. 2010. "The Ecological and Evolutionary Consequences of Clonality for Plant Mating." *Annual Review of Ecology, Evolution, and Systematics* 41 (1): 193–213. https://doi.org/10.1146/annurev.ecolsys.110308.120258.

Wetzel, William C., Brian D. Inouye, Philip G. Hahn, Susan R. Whitehead, and Nora Underwood. 2023. "Variability in Plant–Herbivore Interactions." *Annual Review of Ecology, Evolution, and Systematics* 54 (1): 451–74. https://doi.org/10.1146/annurev-ecolsys-102221-045015.

Wilson, Carol A., Justin Padiernos, and Yuval 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 (1): 35–46. https://doi.org/10.12705/651.3.

HerbVar Publications

A .bib file of all HerbVar presentations is available for download here.

This will eventually have a list of all presentations (eg talks or posters at meetings) by HerbVar Members.

Harper, John L et al. 1977. "Population Biology of Plants." *Population Biology of Plants.*

Herben, Tomáš, and Jitka Klimešová. 2020. "Evolution of Clonal Growth Forms in Angiosperms." *New Phytologist* 225 (2): 999–1010. https://doi.org/10.1111/nph.16188.

Pan, Vincent S., and William C. Wetzel. 2024. "Neutrality in Plant–Herbivore Interactions." *Proceedings of the Royal Society B: Biological Sciences* 291 (2017): 20232687. https://doi.org/10.1098/rspb.2023.2687.

SAPIR, YUVAL, and AVI SHMIDA. 2002. "Species Concepts and Ecogeographical Divergence of Oncocyclus Irises." *Israel Journal of Plant Sciences* 50 (January): 119–27. https://doi.org/10.1560/DJXH-QX0M-5P0H-DLMW.

The Herbivory Variability Network* †, M. L. Robinson, P. G. Hahn, B. D. Inouye, N. Underwood, S. R. Whitehead, K. C. Abbott, et al. 2023. "Plant Size, Latitude, and Phylogeny Explain Within-Population Variability in Herbivory." *Science* 382 (6671): 679–83. https://doi.org/10.1126/science.adh8830.

Vallejo-Marín, Mario, Marcel E. Dorken, and Spencer C. H. Barrett. 2010. "The Ecological and Evolutionary Consequences of Clonality for Plant Mating." *Annual Review of Ecology, Evolution, and Systematics* 41 (1): 193–213. https://doi.org/10.1146/annurev.ecolsys.110308.120258.

Wetzel, William C., Brian D. Inouye, Philip G. Hahn, Susan R. Whitehead, and Nora Underwood. 2023. "Variability in Plant–Herbivore Interactions." *Annual Review of Ecology, Evolution, and Systematics* 54 (1): 451–74. https://doi.org/10.1146/annurev-ecolsys-102221-045015.

Wilson, Carol A., Justin Padiernos, and Yuval 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 (1): 35–46. https://doi.org/10.12705/651.3.