STANDARD OPERATING PROCEDURE: HRB – Plant Pressing, Mounting, and Labeling (Herbarium Techniques)

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	08/31/2016	ECO-03848	Initial release
B	03/21/2017	ECO-04517	• Updated document formatting. • The list of data to collect with vouchers in the field was updated to match the list in the plant diversity protocol.
C	03/16/2018	ECO-05357	• The list of data to be collected with vouchers was updated to match the list in the plant diversity protocol, the plant tissue SOP, and the voucher collection mobile application. • The content of the document 'NEON Herbarium Specimen Label & Annotation Generation' was included in this document for increased clarity and cohesion.
D	01/29/2018	ECO-05958	Updates to voucher collection information and barcode directions as well as some clarity and grammar.
E	01/18/2022	ECO-06711	• Updated to new template (NEON.DOC.002626) • Added SOP A, Preparing for Sampling • Removed SOP E in an effort to keep content focused on SOP • Removed the suggested use of paradichlorobenzene in herbarium cabinets.
F	08/01/2022	ECO-06853	• Updated the document title to reflect application across NEON subsystems, not just TOS.

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1 DESCRIPTION

1.1 Purpose

The purpose of voucher specimens is to provide a permanent record of the NEON naming convention, use of authorities, validation, training, and a means to track taxonomic naming conventions through time. Plant tissue and associated vouchers will also be collected and stored at the archive facility to enable the ecological community to conduct a variety of genetic investigations.

There are four reasons for collecting plant specimens:

1. **Identification at the Domain Support Facility.** These specimens – morphospecies –
    a. Could not be immediately identified in the field
    b. Were collected to be identified in the lab
    c. Will be pressed for preservation according to this SOP if they can't be identified within a few days after collection.

    Because these specimens will be identified at the Domain Support Facility and not included in the reference herbarium, they should be entered in the morphospecies application and labeled in the field, but not collected with the specimen collection application or labeled with a barcode. When the individual has been identified, update morphospecies table, and discard the specimen. When back in the lab, it might be decided that a specimen should be sent for external identification and/or accessioned in the reference herbarium.

2. **Identification by external expert.** Morphospecies that NEON staff can't identify with available resources (e.g. keys, microscopes, regional herbaria) can be sent to contracted experts for identification. These will be pressed, dried, provided with a label, and prepared for shipment following the field season. **These specimens should not be mounted**. Shipping requires a sampleID, so these specimens should be entered in the TOS Plant Vouchers Fulcrum application and a Type II barcode attached to the specimen label.

3. **Inclusion at Domain Support Facility reference herbarium.** These specimens – reference herbarium vouchers - will be used for reference and teaching. They should be pressed, dried, identified, mounted, and labeled. A Type I or Type II "field" barcode can be applied at the time of collection. A Type II "archive" barcode should be associated with the voucher. A quality specimen might require the collection of two individuals should identification require destruction of the sample (e.g., flower and/or ovary dissection). Initially voucher 20 to 200 of the most common (approximately present on > 1/3 plots) and challenging to identify species found within plots at each NEON site. In some cases it will be optimal to collect multiple individuals in different circumstances, particularly when morphology changes across habitats or in response to disturbance (e.g., fire or browsing). Specimens specifically collected for inclusion in the reference herbarium should be collected with the TOS Plant Vouchers Fulcrum application and a Type II barcode attached to the specimen label.

4. **Archive at external facility.** These specimens – archive vouchers - should be pressed, dried, provided with a label, and identified if possible. Terrestrial plant target collection lists by site are available on Sharepoint linked from the Sampling Support Library (SSL). Specimens should be collected with the TOS Plant Vouchers Fulcrum application. A Type I or Type II "field" barcode can be applied at the time of collection. A Type II "archive" barcode should be associated with the voucher prior to shipping. Archive vouchers should be dried and pressed but should not be mounted. They must be of archival quality, collected when fertile and vegetative parts are present, and pressed such that diagnostic parts (e.g. both sides of leaves) are visible.

Numbers three and four above are not mutually exclusive. Some specimens will be sent for identification and then sent to an archive facility.

1.2 Scope

This document provides a change-controlled version of an Observatory procedure. Documentation of content changes (i.e. changes in particular tasks or safety practices) will occur via this change-controlled document, not through field manuals or training materials.

1.3 Applies To

The procedure described in this document is used in the following protocols:

Doc #	Title
NEON.DOC.014042	TOS Protocol and Procedure: Plant Diversity Sampling
NEON.DOC.014040	TOS Protocol and Procedure: Plant Phenology
NEON.DOC.000987	TOS Protocol and Procedure: Measurement of Vegetation Structure
NEON.DOC.001024	TOS Protocol and Procedure: Canopy Foliage Chemistry and Leaf Mass Per Area Measurements

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

Applicable documents contain higher-level information that is implemented in the current document. Examples include designs, plans, or standards.

AD[01]	NEON.DOC.004300	EHS Safety Policy and Program Manual
AD[02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD[03]	NEON.DOC.001155	NEON Training Plan
AD[04]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[05]	NEON.DOC.050724	Domain Chemical Hygiene Plan and Biosafety Manual

2.2 Reference Documents

Reference documents contain information that supports or complements the current document. Examples include related protocols, datasheets, or general-information references.

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.002652	NEON Data Products Catalog
RD[07]	NEON.DOC.014040	TOS Protocol and Procedure: Plant Phenology
RD[08]	NEON.DOC.000987	TOS Protocol and Procedure: Measurement of Vegetation Structure
RD[09]	NEON.DOC.001025	TOS Protocol and Procedure: Plot Establishment
RD[10]	NEON.DOC.014042	TOS Protocol and Procedure: Plant Diversity Sampling
RD[11]	NEON.DOC.001024	TOS Protocol and Procedure: Canopy Foliage Sampling
RD[12]	NEON.DOC.004257	NEON Standard Operating Procedure (SOP): Decontamination of Sensors, Field Equipment and Field Vehicles
RD[13]	NEON.DOC.005224	NEON Protocol and Procedure: Shipping Ecological Samples and Equipment

2.3 Acronyms

All acronyms used in this document are defined in RD[01].

2.4 Definitions

Annotation Label: subsequent identifications and notes added to a herbarium specimen when researchers use the specimen for study, the confirmation of the initial identity of the specimen by an expert botanist, corrections to the identification, and voucher use history.

Cladode: photosynthetic branch / cactus pad.

Collection Label: label attached to a specimen that documents the specimen's metadata.

Collection Number: unique number given to each specimen collected by a particular person. Collection numbers are given sequentially, with the first specimen a person ever collected being "1", second being "2", and so on until the end of a person's collecting career.

Fragment Packet: folded paper envelope glued to a herbarium sheet and used to store loose plant specimen parts.

Fulcrum: Software tool used to create NEON electronic data entry applications.

Genus folder: heavy paper folder used to store all herbarium specimens in one genus.

Glochid: tiny hair-like spines on Cactaceae branches originating from the areoles.

Graminoid: grasses and grass-like organisms; includes the families Poaceae (true grasses), Cyperaceae (sedges), and Juncaceae (rushes).

Herbarium: archive of preserved plant specimens organized alphabetically or taxonomically and used for reference and to document taxa to which names are attached.

Morphospecies: plants that appear morphologically to be the same or very closely related but that are not yet determined to species.

Node: portion of stem where leaves attach.

Parenchyma: succulent tissue inside cactus stems.

ServiceNow: Software tool used for problem/incident tracking and resolution.

Species Folder: floppy paper folder used to store all herbarium specimens of one species.

Taxon (*plural* **Taxa**): monophyletic group of plants.

Vasculature: system of vessels through which a plant transports liquids (xylem and phloem).

Ventilator: sheet of corrugated cardboard used in the pressing, organization, and transportation of herbarium specimens.

Voucher Specimen: biological specimen to which names are attached.

3 SAFETY

This document identifies procedure-specific safety hazards and associated safety requirements. It does not describe general safety practices or site-specific safety practices.

Personnel working at a NEON site must be compliant with safe field work practices as outlined in the Operations Field Safety and Security Plan (AD[02]) and EHS Safety Policy and Program Manual (AD[01]). Additional safety issues associated with this field procedure are outlined below. The Field Operations Manager and the Lead Field Technician have primary authority to stop work activities based on unsafe field conditions; however, all employees have the responsibility and right to stop their work in unsafe conditions.

3.1 Toxic Plant Safety

Toxic plants will be collected for identification and for vouchering. Many Domains will have lists of potentially harmful plants. Instructions for handling *Toxicodendron* species are included in this document (APPENDIX D). Personnel should also be made aware of local plant taxa that are not poisonous but can cause acute discomfort such as *Cnidoscolus stimulosus*, *Urtica dioica*, and *Laportea canadensis* and handle them accordingly.

3.2 Cactaceae Safety

The potential for injury exists when working with cacti due to the prevalence of spines. As a precautionary measure, avoid making contact with cacti. Always wear personal protective equipment such as leather boots, long pants, long sleeves, and gloves, and remain cognizant of where you walk.

Most cactus-inflicted wounds should be treated like any other scratch or puncture, that is, cleaned and then bandaged if necessary. However, spines often break off upon contact and must be picked out using either a comb or tweezers prior to treating the affected area.

Glochids that penetrate the skin may be difficult to extract because of their barbed shafts. To remove glochids, use tweezers or adhesives. Treat any remaining glochids as a wound, keep the area clean and covered or it may develop dermatitis.

If a *Cylindopuntia* sp. segment becomes attached to you, pull the segment off yourself by using a comb or solid object (i.e. Leatherman, rocks, etc.). Symbiotic bacteria often live on cactus spines which can instantly cause inflammation, but it will go away on its own within a few days.

3.3 Chemical Safety

Safety Data Sheets (SDS) are available for the following chemicals used in this work: ethanol. Whenever chemicals are used, follow requirements in the specific Safety Data Sheet and of the site-specific Chemical Hygiene and Biosafety Plan (AD[03]), to include disposal of used chemicals.

4 PERSONNEL

4.1 Training Requirements

All technicians must complete required safety training as defined in the NEON Training Plan (AD[04]). Additionally, technicians must complete procedure-specific training for safety and implementation of this procedure as required in Field Operations Job Instruction Training Plan (AD[05]).

4.2 Specialized Skills

While any technician carrying out plant-related protocols may collect a specimen in need of identification, only technicians fully versed in botany should be assigned to collect vouchers for the herbarium. Technicians participating in voucher processing in the lab must have at least a rudimentary understanding of plant morphology and display attention to detail and great patience. Technicians assigned to press and mount specimens must be fully trained by a qualified coworker in order to produce quality, useful vouchers.

5 CONTINGENCIES AND NOTES

5.1 Sampling Frequency and Timing

Most voucher specimens will be collected during the first two sampling seasons in order to establish a collection. Specimens should be collected when fertile parts are present. It may take multiple field seasons to amass a representative sample of the Domain's flora. Voucher specimens will be collected opportunistically for the remainder of the Observatory's life to document changes in floral composition at the site.

5.2 Criteria for Determining Onset and Cessation of Sampling

Collection should begin during a Domain's first year of sampling and continue opportunistically to build a robust Domain herbarium.

5.3 Timing for Laboratory Processing and Analysis

Ideally, specimens should not be left in the refrigerator for more than two days. Specimens will begin to decompose even while refrigerated; however, some specimens will be more prone to this than others (Asteraceae spp. versus graminoids). Closely monitor your refrigerated specimens if immediate identification is not possible and they are refrigerated for more than two days; they can also be placed in the press, dried, and identified at a later date. Identification often requires a variety of dichotomous keys, a dissecting microscope, a dissecting kit, and an herbarium with voucher specimens for verification. Any specimen destined for the internal reference herbarium should be placed in a -80°C freezer for approximately two weeks for decontamination after it is completely dried. Specimens destined for an external archive may also require freezing pending contract requirements.

5.4 Estimated Time

Approximately 18 days are required to produce a single voucher for the herbarium:

- Collect plant (three minutes), press plant (three minutes)
- Dry plant (three days)
- Generate label with Herbarium and Determination Label Generator accessed via Sharepoint (5 minutes)
- Quality control review Fulcrum data and label
- Glue plant to mounting paper (for vouchers not shipped to external archive; 5-15 minutes)
- Dry glued specimen (one day)
- Freeze specimen (two weeks)
- Insert specimen into herbarium (5 minutes)

6 STANDARD OPERATING PROCEDURES

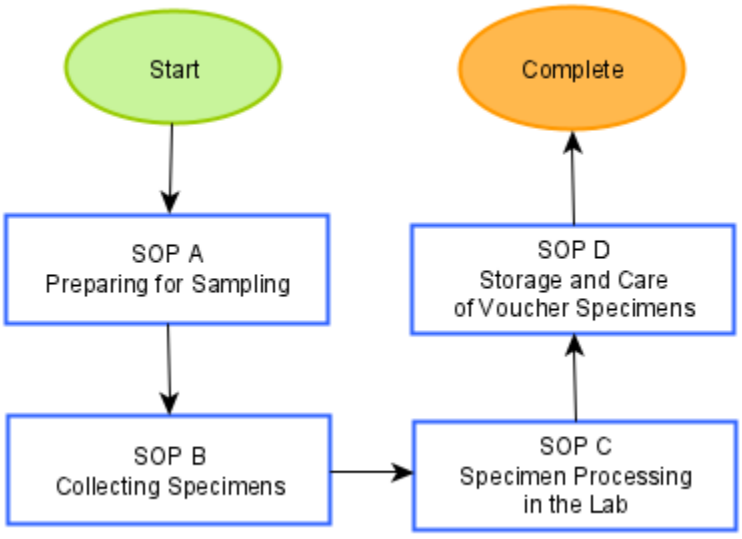


Figure 1. Overview of the standard operating procedures (SOP) that describe plant pressing and mounting.

The Standard Operating Procedures (SOPs) in this document provide directions, contingency plans, sampling tips, and best practices for the handling, care, and preservation of plant material (**Figure 1**). The value of NEON data hinges on consistent implementation of this protocol across all NEON domains, for the life of the Observatory. It is therefore essential that field personnel carry out this protocol as outlined in this document. In the event that local conditions create uncertainty about carrying out these steps, it is critical that technicians document the problem and enter it in NEON's problem tracking system.

SOP A Preparing for Sampling

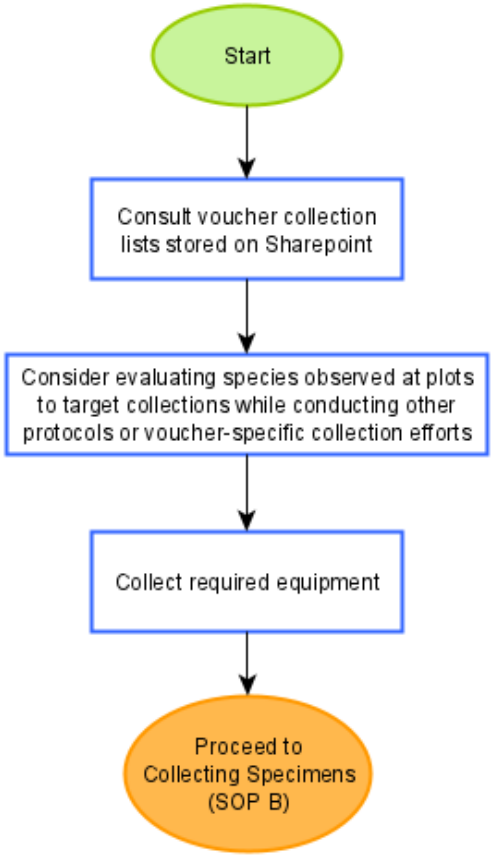


Figure 2. Flow diagram of activities to be completed prior to going to the field.

In addition to collecting and preparing required field equipment, including charged electronics, labels, and notebooks, several activities can facilitate targeted collection in the field (**Figure 2**).

1. For the archive voucher (again, those specimens destined for the external bioarchive) collections, compare site- and year-specific voucher collection lists on Sharepoint linked from the Sampling Support Library and to species that have been collected for the archive to create a list of species that need to be vouchered.

2. Consider evaluating species documented in plant diversity and vegetation structure data to better plan locations where species have been located. If it is not appropriate to harvest specimens from the plot, areas near a plot or with similar conditions could be searched.

3. For the reference vouchers (those specimens to be housed at the reference herbarium at the domain support facility) it can be useful to review the lists (in Fulcrum or NEON Portal data or otherwise is reference herbarium vouchers have not been uploaded) of species that have been

collected such that species particularly useful for training might be collected, or to ensure time is not spent collecting a species already represented by multiple reference vouchers.

SOP B Collecting Specimens

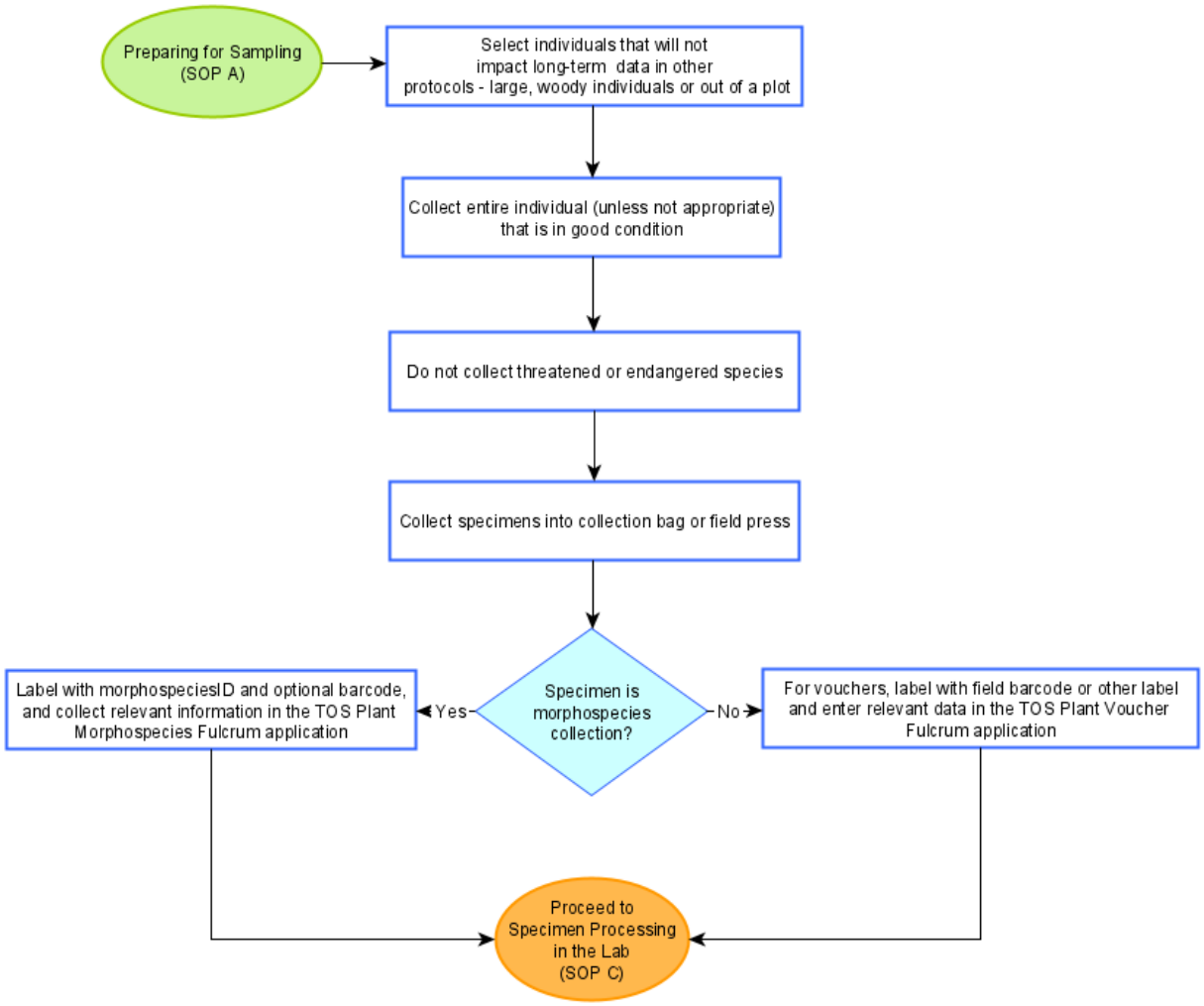


Figure 3. Workflow for collecting voucher specimens in the field.

B.1 Field Collection

These guidelines and steps should be followed when collecting specimens (**Figure 3**):

1. For terrestrial systems, select specimens that are outside of the 20x20 m or 40x40 m plot if site-specific permit allows, or from within plots but outside the 20x20 m area where diversity observations are made. For aquatic systems, collect samples from within the sampling reach, either outside the transect or from within a quadrat in a biomass collection.
2. Select plants in good condition, free of damage from insects and/or disease unless damage or disease is representative of the population as a whole.

3. If possible, all parts of a plant should be collected: the roots, rhizomes, stems, flowers, fruits, and basal leaves.
    - If necessary for identification, take extra bits of particularly informative organs, such as inflorescences, so that you will have plenty to dissect.
    - If a species has heteromorphic leaves, make sure to collect leaves of all shapes/sizes.
    - Young leaves, basal rosettes, stump sprouts, and both mature and immature twigs are very informative and important to include.
    - Take care when digging below ground organs out, as they are often needed for identification. A sharp object like a trowel or hori hori works well.
4. Collect seedlings and saplings as they often look different than their mature counterparts and are a useful tool for the teaching collection.
5. Do not collect the following:
    a. A meager specimen
    b. Individual plants that significantly impact the population (i.e., rare species).
    c. Listed or protected species. It is important to bring a list and be familiar with protected plant species in the field to double check that one does not collect a protected species.
6. If the species you want cannot be collected because it is a protected species, collecting it would harm its population, or a specimen cannot be found outside of the plot, take pictures of all plant parts as if making a collection. For small features make sure the camera is sufficiently close to resolve minute details of plant parts.
7. Place all specimens of a single species from one locality into one collection bag and,
    a. For unknown/morphospecies: write the morphospeciesID on a specimen label included in the bag and attached to the specimen.
    b. For voucher specimens: record information in step #8 within the TOS Plant Vouchers Fulcrum application, and label specimen with the voucher sample ID (see below) and a Type II ‘field’ barcode attached to the specimen label.
8. Record pertinent information. If the specimen is destined for the Domain Support Facility reference herbarium, or external archive, record:
    - **Domain ID**. The domain in which the specimen is collected.
    - **Site ID.** The site at which the specimen is collected.
    - **Plot ID** (if applicable). The plot number from which the specimen is collected.
    - **Location** – if not at plot. If the voucher is not collected in (or near) a plot, record coordinates, uncertainty (if available), and elevation.
    - **Date.** YYYY-MM-DD

- **Identified By.** The 'Collector Name', name of the person responsible for recording original occurrence.
- **Recorded By.** The name of individual recording information.
- **Collected By.** The name of the individual who collected the specimen.
- **Sample Tag Number** (e.g., a phenology or vegetation structure tag)**.** Record a NEON sample tag number if present.
- **Taxon ID.** The NEON taxonID to lowest possible taxonomic rank.
- **Taxon ID Remarks.** Notes about the specimen.
- **Identification Qualifier** (if appropriate). The standardized term to qualify the identification of the organism when doubts about taxonomic identity exist.
- **Identification References** (if appropriate). The name of the reference used to identify the specimen.
- **MorphospeciesID** (if appropriate). The temporary name for a specimen not identified to species or lower taxonomic rank.
- **Plant description**. A description of notable specimen characteristics e.g., Very small yellow flowers turning white with age, small lanceolate leaves. Flattened round fruit.
- **Life stage.** The age class of the individual (e.g., 'fruiting', 'seedling').
- **Locality.** Natural language description of the place where the organism was collected, e.g., Blue Mountains, 50m west of summit of Grandfather Mountain.
- **Habitat Description.** A category or description of the habitat in which the specimen occurred.
- **Associated Taxa.** NEON taxonID of plant species associated or found in proximity to the collected specimen.
- **Voucher (Collection) Number**. An identifier given to the specimen at the time it was recorded; typically a collector-specific running number (sometimes called record number).
- **Voucher Sample ID.** This unique number is comprised of the prefix 'pla', site, date, time, collector initials, and collector number, e.g., pla.OAES.20151014.10:30.dtb.V123. The voucher application generates these sample IDs after time and middle initial is entered.

9. Certain plant taxa have characteristics that must be present in order to reliably identify a member to species. Chapter 3 of The Herbarium Handbook contains an extensive list of such taxa (Bridson, 1998). Each Domain will likely need to generate a list, but **Table 1** provides a rudimentary one to start.

Table 1. Plant taxa that may be difficult to identify to species without certain morphological characteristics present.

Taxon	Necessary characteristics
Apiaceae	mature fruits, basal leaves, below-ground organs
Asteraceae	mature fruits, basal leaves (or record lack thereof during flowering/fruiting)
Astragalus	mature fruits
Boraginaceae	mature seeds
Brassicaceae	mature fruits and often petals, petal color
Caryophyllaceae	mature seeds
Cyperaceae	mature fruits, below-ground organs
Juncus	mature seeds
Lamiaceae	record odor of fresh, crushed leaves
Myrtaceae	include photographs or a thorough description of bark, juvenile leaves
Orchidaceae	flowers
Poaceae	mature flowers, below-ground organs such as roots and rhizomes
Polygonaceae	mature flowers or fruit
Rosa	normal, woody branches (not vigorous young shoots), flowers, stem habit (e.g. arching, climbing, trailing)
Rubus	stem habit (e.g. arching, trailing, ascending)
Viola	mature flowers or fruits

10. Palms and certain other trees can be difficult to sample because their leaves are held far out of human reach. The Missouri Botanical Garden (Liesner, 2016) has helpful instructions for sampling tall plants, which includes the use of tree trimming poles and shotguns. If leaves and fertile parts of a species can only be accessed with such tools, collect them in conjunction with canopy foliar chemistry sampling (RD[11]) efforts, or do not attempt to collect the specimen.
11. Cactus collection requires special care. Note the following characteristics when you collect because these characteristics tend to change as cacti dry:
    a. Are areoles grooved or not
    b. Position of flowers on the plant (tip of the stem, all along the stem, etc.)
    c. Flower color (including the color of each of the various parts)
    d. Petal margin detail
    e. Flower height and width
    f. Fruit dimensions
    g. Fruit color and fleshiness
    h. Spine color, or number of hooked spines prior to pressing (Clover, 1952).

Use a sturdy set of tongs to collect cacti with minimal injury to oneself and the specimen. Use gloves only when working with specimens that do not have glochids, as the glochids will work their way into the gloves (Clover, 1952). When working with *Opuntia* and other genera with glochids, use a fold of cardboard (or small plastic cutting board or similar), or a large rock along with tongs to collect specimen parts (Clover, 1952) (Griffiths, 1907). See Appendix A for more information about collecting Cactaceae sp.

B.2 Refreshing the Sample Kit

Be sure to have sufficient plastic bags, voucher slips - pertinent fields can be preprinted -, and permanent markers for the next field sampling effort.

SOP C Specimen Processing

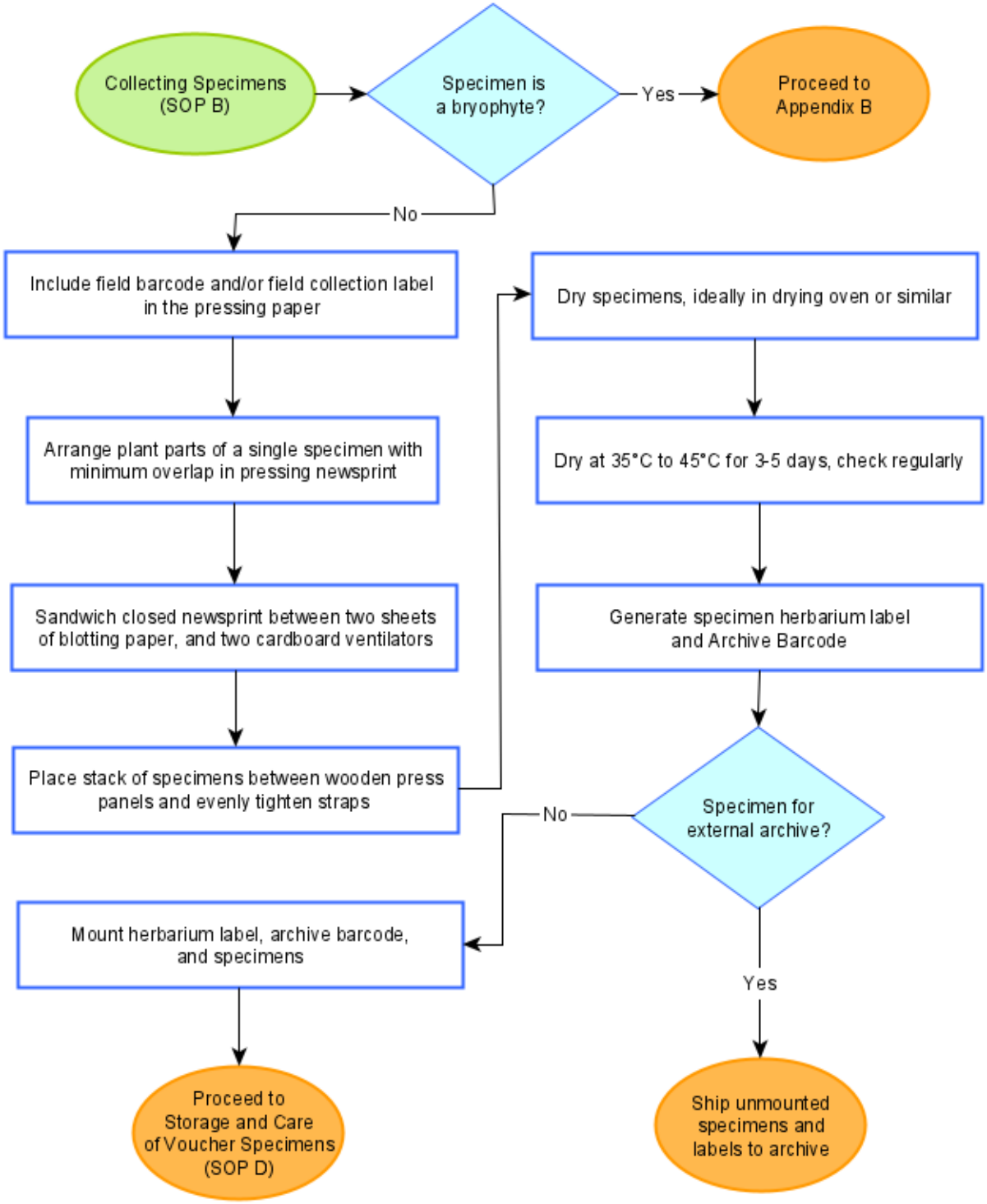


Figure 4. The workflow for handling voucher specimens in the lab.

The processing component of plant collection for voucher or morphospecies includes: plant pressing, drying, identification, and processing of a subset of collected species for voucher specimens (**Figure 4**). The exact order and need for each step with each specimen will depend on scheduling, available time, and the objectives for the particular specimen.

Some species will come from the field and be identified fresh (without pressing), ideally within two days of collection. Some specimens will take longer to decompose and if it is preferred to identify them while fresh, they can remain in the refrigerator for longer periods of time with careful monitoring. If the specimen was destroyed during identification or was not intended for vouchering, do not save and press the specimen. Not every unknown plant species must be vouchered and submitted to the reference herbarium or the external archive.

Some species will be collected, and the botanist will not have time to identify them prior to degradation or will be unable to identify the specimen. These specimens should be pressed, dried in a well-ventilated location, frozen at -80°C for approximately two weeks (to kill any insect pests), stored in the closed herbarium cabinet, and identified at a later date, either by the botanist with the help of an herbarium and/or books, or by sending them to an expert.

Other specimens will be collected specifically for vouchering at a NEON reference herbarium or the external bioarchive facility. These specimens should be treated with extra care to preserve diagnostic parts. All will be pressed, dried, provided with a collection label, and decontaminated in the freezer for approximately two weeks at -80°C. Those specimens destined for the Domain Support Facility's reference herbarium should be mounted, whereas those destined for an external archive should remain unmounted in their pressing paper and will be shipped to the archive.

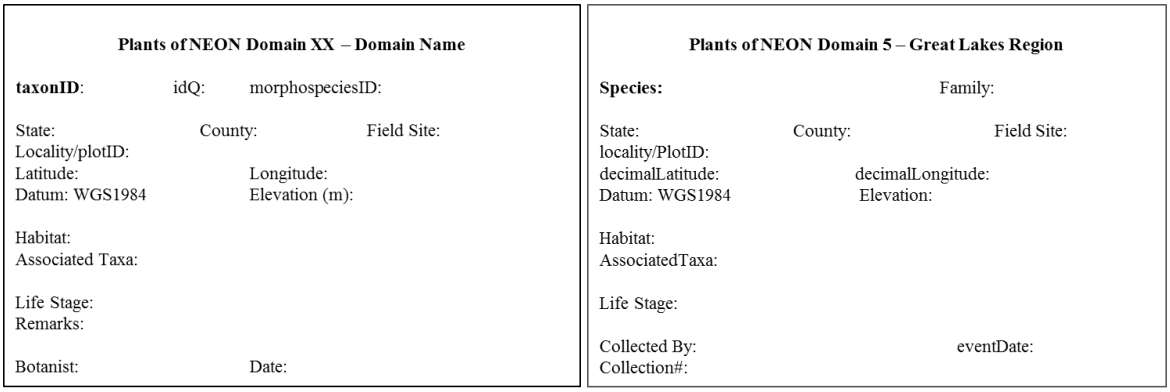
C.1 Pressing

Plant pressing can occur in the field or in the lab. Pressing in the lab is more common as it prioritizes efficient use of available field hours and does not require that the press be carried in the field. Individuals must be handled and transported with care to avoid mangling of vulnerable parts (e.g., flowers), kept cool during transport if possible, and either pressed upon arrival at the lab or stored at 4°C (i.e., refrigerated) for no longer than two to four days. Pressing plants in the field can better preserve fragile parts and result in better vouchers. However, collections are often made in conjunction with other protocols in the field, and the time required for field pressing and space for carrying extra equipment may not be available. The following steps apply to lab and field pressing, but the field pressing order of operations require slight modification (e.g., labels will need to be generated upon return to the lab).

Do not press bryophytes; separate instructions for bryophytes are provided (Appendix B).

Plants are pressed and dried in standard plant presses and newspaper as follows:

1. Before pressing plants, make sure the pressing newspaper is the same size as the mounting paper (11.5 by 16.5 inches).
    - This will ensure that the dried specimens will fit on the herbarium paper on which they may eventually be mounted, and that the pressing newspaper containing the specimen will fit in the herbarium cabinet while awaiting identification and/or mounting.
    - Tabloid newspaper may be ideal for pressing plants since it is often already the appropriate size.
    - If your paper is larger than 11.5 by 16.5 inches cut it down to size prior to pressing.
2. Place a field collection label with all pertinent collection information inside the pressing paper. This label should only be used as a temporary means to track sample, NOT as the final label for herbarium vouchers. See B.3 for final herbarium label instructions. Include the field barcode collection label with the following (**Figure 5**):
    - **Collector name**. Name of the person responsible for recording original occurrence.
    - **TaxonID.** The NEON taxonID to lowest possible taxonomic rank.
    - **MorphospeciesID** (if appropriate). The temporary name for a specimen not identified to species or lower taxonomic rank.
    - **Voucher Sample ID**. This unique identifier provides a link to the information about the specimen for labeling or shipping to an external archive. This unique number is comprised of the prefix 'pla', site, date, time, collector initials, and collector number, e.g., pla.OAES.20151014.10:30.dtb.V123. The voucher application generates these sample IDs.



Plants of NEON Domain XX – Domain Name

taxonID: idQ: morphospeciesID:

State: County: Field Site:
Locality/plotID:
Latitude: Longitude:
Datum: WGS1984 Elevation (m):

Habitat:
Associated Taxa:

Life Stage:
Remarks:

Botanist: Date:

Plants of NEON Domain 5 – Great Lakes Region

Species: Family:

State: County: Field Site:
locality/PlotID:
decimalLatitude: decimalLongitude:
Datum: WGS1984 Elevation:

Habitat:
AssociatedTaxa:

Life Stage:

Collected By: eventDate:
Collection#:

Figure 5. Blank field collection label; edit as needed for your domain and site. This label should only be used as a temporary means to track samples, NOT as the final label for herbarium vouchers. Sample field collection label from D05 (courtesy of Jill Pyatt).

3. Within the fold of one sheet of pressing newspaper, carefully arrange plant parts of a single specimen with minimum overlap (**Figure 6**):
    a. Turn over some leaves or part of a single large leaf to show underside.
    b. Ensure that at least one node is visible. The more nodes left visible the better!
    c. Open some flowers to show both the top and underside to illustrate the arrangement of flower parts.
    d. If a specimen has an excess of leaves and/or branches, some may need to be removed in order to expose other more informative plant organs (**Figure 6**).
    e. Squash fleshy fruits on the page or slice them until they are a manageable thickness (around one centimeter or less). Examples of fruits that may be treated thus are osage oranges, cherries, and blueberries.
    f. Do not press especially large or lignified fruits (e.g., acorns, chestnuts) or seed cones. Instead, give them a tag with complete collection information and put them in the drying oven until desiccated. Osage oranges and large citrus may also be preserved in this fashion; slicing them in half will reduce drying time.
    g. Place dry, loose seeds and fruits in fragment packets and include inside the pressing paper so parts do not fall out and get lost (Appendix D).
    h. Try to make the specimen as flat and thin as possible. This may mean cutting thick branches lengthwise and cutting things like limber pine's needles off. Also, it is beneficial to cut off spur branches that won't be visible once pressed.
    i. Some specimens will be too large to press in a single sheet of newspaper. Distribute these into as many sheets as necessary and label with "1 of 3", "2 of 3", and so on. Be sure to include duplicate collection information with each sheet.
    j. Certain plant taxa, such as cacti, aquatics, palms, and bamboos require special treatment (Appendix B).

Figure 6. A properly pressed specimen. Note the plant does not stick out of the newspaper; the newspaper has been cut to 16.5 x 11.5 inches so it fits perfectly in the plant press, and a printed label on acid-free paper is inserted into the center fold.

4. Close newspaper and sandwich between two sheets of blotting paper. Blotting paper will not always be necessary but is helpful in especially humid environments and when pressing very wet or fleshy plants such as succulents and aquatics. Place newspaper-blotting paper sandwich between two cardboard ventilators. Make sure that the ventilators have fully expanded corrugations so that air can pass through them (**Figure 7**).

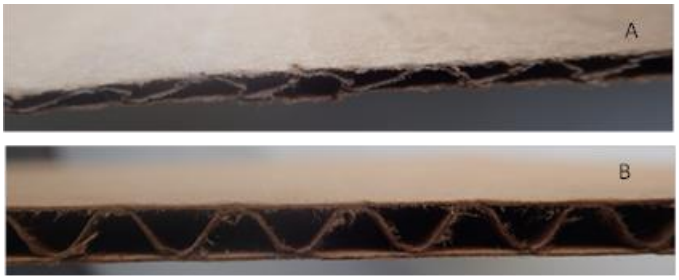


Figure 7. Ventilators with crushed corrugations (A) and fully expanded corrugations (B). Ventilators with crushed corrugations should not be used to press and dry specimens because they cannot channel air to dry the plant; instead, use ventilators with fully expanded corrugations. Ventilators with crushed corrugations are ideally suited for use during mounted specimen glue drying and as family separators in the herbarium cabinet.

5. Place the stack of specimens, blotting paper (optional), and ventilators between the wooden plant press panels (**Figure 8**) and tighten the straps as much as possible. It can be helpful to kneel on the press to tighten. Make sure the press is even.

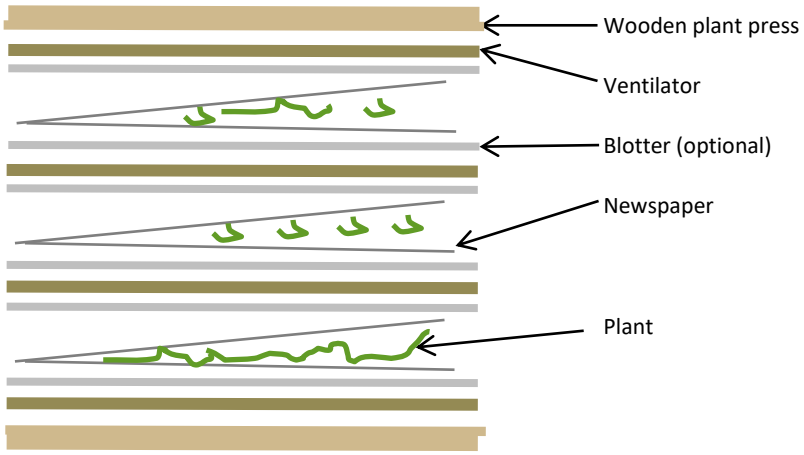


Figure 8. Sequence of layers employed during plant pressing. Use this order: wooden plant press, ventilator, blotter (optional), newspaper containing plant, blotter (optional), ventilator, blotter (optional), newspaper containing plant, blotter (optional), etc., wooden plant press.

C.2 Drying

Dry specimens in the press. It is best to immediately place the press in a drying oven, as even graminoids can start to discolor if not dried rapidly. If a drying oven is unavailable, grasses, sedges, and other small specimens may dry without damage in a well-ventilated part of the lab with a fan running next to them, as long as the blotters are changed out. Larger and fleshy or aquatic plant specimens will require a drying oven to prevent molding.

Plant presses should be put in a 35°C to 45°C drying oven for three to five days. Ensure that the temperature does not exceed 80°C, as high temperatures will cook the plants and damage molecular structures. Rather, we want to keep slightly warm, dry air moving through the press.

Check the press daily to cinch down the straps and change out blotters.

If the supply of ventilators runs low, cut more out of corrugated cardboard boxes, making sure that the corrugations run along the width of the sheet and not the length. This will allow more air to pass through the ventilator (**Figure 9**).

Ventilators with crushed corrugations (**Figure 3**) no longer allow air to pass through them should be removed from the plant presses and repurposed for mounting or herbarium cabinet storage.

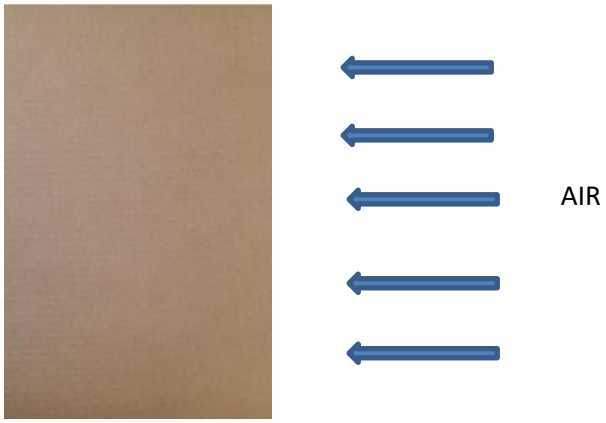


Figure 9. Illustration showing how corrugations in a ventilator run along its width, not along its length, in order to facilitate air movement.

C.3 Label Generation

Herbarium specimens to be accessioned in the Domain Support Facility and for the external archive have a standardized label. To facilitate the creation of labels, a Shiny application has been developed. The information needed for the label can be tracked by the 'voucherSampleID'. Specimens destined for an external archive or identification should not be labeled or mounted.

In this document the term *taxon* (plural *taxa*) is used to refer to a scientific name at any rank. Therefore, the term *species* is used literally to refer to the taxonomic rank below Genus.

1. Label Format

    - Labels should not exceed 5.5" in width and 3.5" in height. Smaller is acceptable. The most critical aspect is whether all the relevant information can fit onto the label. A larger label may be used if necessary, though only the height should be increased—not the width. Use the font type Times serif 12 point. Smaller text may be used down to 10 point. Label metadata should be left-justified, except for the date, which is right-justified and the header and footer, which are centered. The entire scientific name including the author should be italicized so that this information will be more obvious on the label. The scientific name (excluding author information) should be in bold face as well.
    - The following header format should be used:

        Plants of NEON Domain # – Domain Name

        Be sure to bold this header. An example for a site in domain 9 would be:

        Plants of NEON Domain 9 – Northern Plains

    - The following footer should be used:

        Collected as part of the National Ecological Observatory Network

2. Data To Be Included

   A comprehensive list of potential data to be included on the label is outlined in **Table 3** (note there are required, preferred, and optional data). Required data are outlined below with a description for formatting requirements.

   - Header: NEON Domain # and Name
   - Family: Scientific name, in all caps
   - Scientific name, including author, all italicized, with name in bold
   - Location, hierarchically arranged from general to specific:
     - Country
     - State/Province
     - County
     - NEON site, spelled out
     - NEON plot number, IF applicable
     - Decimal Latitude and Longitude
     - Geodetic Datum
     - Elevation, in meters
   - Habitat description
   - Associated species, italicized, preceded by With
   - Notes about the plant
   - Collector name with collection number
   - Date
   - Footer

3. Spatial Separation of Data

   The data on the label should be spatially separated by type of information in the following categories:

   - Header
   - Family and scientific name
   - Location
   - Habitat description and associated species
   - Notes about the plant
   - Collector name with collection number and date
   - Footer

Table 2. Data to be included on labels. DwC is the abbreviation for Darwin Core. The terms are based on the Darwin Core metadata standard for biodiversity informatics (version 2011-10-26, http://rs.tdwg.org/dwc/terms/index.htm#theterms).

Necessity	Standard	Term	Plant Diversity Field Name	Description	Example	Information Type	Notes
Required	NA	header	domain	The NEON domain name and number where collected	10 - Central Plains	Location	
Required	DwC	family		The name of the family in which the organism is classified. (e.g., “Fagaceae”)	FABACEAE	Scientific name	
Required	DwC	genus		The name of the genus in which the organism is classified. (e.g., “Quercus”)	*Dalea*	Scientific name	
Required	DwC	specificEpithet		The specific epithet of the scientific name applied to the organism. (e.g., “agrifolia”)	*candida*	Scientific name	Required only if known
Required	DwC	infraspecificEpithet		The infraspecific epithet of the scientific name applied to the organism. (e.g., “oxyadenia”)	*subsp. oligophylla*	Scientific name	Required only if known. subsp. or var. should proceed this if present
Required	DwC	scientificNameAuthorship		The authorship information for the scientificName formatted according to the conventions of the applicable nomenclaturalCode (ICBN). e.g. "(Torr.) J.T. Howell".	Pursh	Scientific name	
Optional	DwC	identificationQualifier		A brief phrase or a standard term to qualify the identification of the organism when doubts have arisen as to its taxonomic identity. (e.g., "cf.", "aff.")	cf.	Scientific name	
Required	DwC	country		The full, unabbreviated name of the country. (e.g., “United States”)	United States	Location	
Required	DwC	stateProvince		The full, unabbreviated name of the state, province. (e.g., “North Carolina”)	North Dakota	Location	
Required	DwC	county		The full, unabbreviated name of the county or parish. (e.g., “Avery“)	Stutsman	Location	
Required	NA		site	The NEON site where collected	Central Plains Experimental Range	Location	

Necessity	Standard	Term	Plant Diversity Field Name	Description	Example	Information Type	Notes
Preferred	NA		plotID	The NEON plot where collected	CPER_001	Location	Plot identifier (4-character site code _XXX)
Optional	DwC	locality	remarks	The specific natural language description of the place where the organism was collected. (e.g., “Grandfather Mountain”)	Killdear Mountain	Location	
Required	DwC	decimalLatitude	decimalLatitude	The latitude of the geographic center of a location where collected, in decimal degrees. Positive values are North of the Equator, negative values are South of the Equator (-90 to 90). (e.g., 33.4558)	46.386676 N	Location	
Required	DwC	decimalLongitude	decimalLongitude	The longitude of the geographic center of a location where collected, expressed in decimal degrees. Positive values are East of the Greenwich Meridian, negative values are West of the Greenwich Meridian (-180 to 180). (e.g., -88.7884)	-103.302402 W	Location	
Required	DwC	geodeticDatum		The geodetic datum to which the latitude and longitude refer. If not known, use "not recorded". (e.g., "WGS84")	WGS84	Location	Recommend WGS84
Preferred	DwC	ElevationInMeters	elevation	The elevation at which the collection or observation was made. (e.g., “1818”)	1068 m	Habitat	
Preferred	DwC	habitat		A category or description of the habitat in which the Event occurred. (e.g. “riparian woodland”)	grassland	Habitat	
Preferred	DwC	associatedTaxa	Associated taxa	A list of identifiers or names of taxa and their associations with the Occurrence.	*Carex siccata*	Associated Species	Preceded by the word With
Optional	DwC	lifeStage		The age class or life stage of the biological individual. (e.g., “seedling”)	flowering	Notes about the plant	

Necessity	Standard	Term	Plant Diversity Field Name	Description	Example	Information Type	Notes
Optional	DwC	eventRemarks		Comments or notes about the Event.	gravelly slope	Location/Habitat	
Required	DwC	recordedBy	Collector name	The name of the primary collector or observer (“T.S. Doode”)	J. Smith	Collector	
Required	DwC	recordNumber	Collection number	An identifier given to the Occurrence at the time it was recorded. Often serves as a link between field notes and an Occurrence record, such as a specimen collector's number.	54	Collector	
Required	DwC	eventDate	eventDate	The date-time in which an organism was collected. (e.g., “1992-03-22”)	2017-07-27	Date	
Required	NA	footer	Project name	Written description of the NEON project	Collected as part of the National Ecological Observatory Network	Entity	

4. Materials Needed

   - Paper: Archival quality 25-100% cotton rag paper can be cut, or precut specimen labels can be used.
   - Printer: Inkjet preferred, but laser may also be used.
   - Glue: A white PVA resin, plasticized, water soluble adhesive should be used.

5. Label Examples

   All label data in figures below are fictitious and are not based on actual specimens or data. **The border outlining the label is for illustrative purposes only. DO NOT print labels with a border around them.**

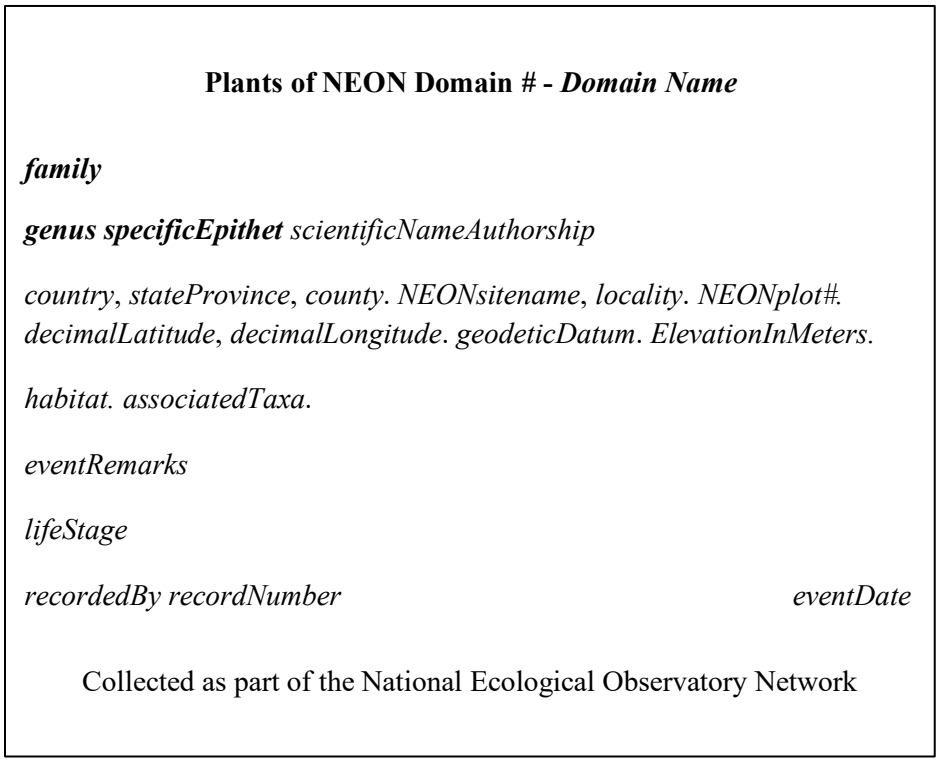


Figure 10. Label showing placement of metadata elements. These terms are defined in Table 3.

Plants of NEON Domain 3 – Southeast

Sabal etonia *Swingle* ex *Nash*

United States, Florida, Putnam County. Ordway-Swisher Biological Station, southwest of Lake Rowan. NEON Plot OSBS_011. 29.673119 N, -82.025924 W. WGS84. 44 m.

Xeric hammock with well drained sandy soils. With *Quercus spp., Pinus palustris*, *Lyonia*, *Serenoa* & *Zanthoxylum*.

Fruiting

I.M. Haybaler 7,987 2014-07-19

Collected as part of the National Ecological Observatory Network

Figure 11. Example label #1.

Plants of NEON Domain 3 - Southeast

Aristida stricta *Michx.* ***var. beyrichiana*** *(Trin. & Rupr.) D.B. Ward*

United States, Florida, Putnam County. Ordway-Swisher Biological Station, east of Melrose. ~100 meters south of Mason Road. NEON Plot OSBS_027. 29.673119 N, -82.025924 W. WGS84. 47 m.

Xeric hammock with well drained sandy soils. With *Diospyros virginiana, Sabal etonia, Zanthoxylum clava-herculis, Osmanthus americanus, O. megacarpus, Garberia heterophylla, Ceratiola ericoides, Ilex vomitoria, Rhynchospora megalocarpa, Dichanthelium spp., & Solidago odora*.

Flowering and fruiting

I.M. Haybaler 7,901 2014-07-19

Collected as part of the National Ecological Observatory Network

Figure 12. Example label #2.

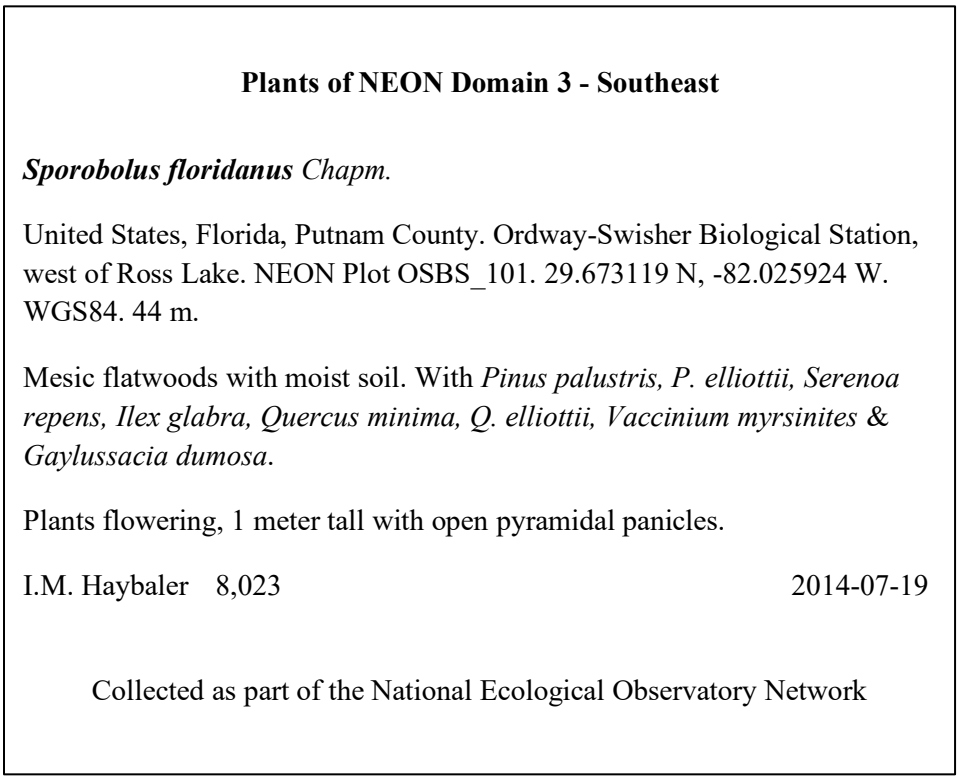


Figure 13. Example label #3.

C.4 Barcode Labels

The 'field' barcode will likely be stuck to the field collection label or the actual specimen and not suitable for a final voucher specimen. An 'archive' barcode should be created in the Fulcrum application in the existing voucher record for each voucher.

- In the case of vouchers destined for the external archive, the archive barcode should be cut out from the barcode source such that it can adhere to the mounting sheet when the specimen is mounted at the bioarchive. If this is difficult, the barcode can be stuck to a blank piece of label paper.
- Vouchers for the reference herbarium should also have an archive barcode created that will be adhered to the mounting sheet during the voucher mounting process.

C.5 Annotation Labels

The scientific value of herbarium specimens is improved by annotation. An annotation label should be used whenever a change, note, or verification to a specimen needs to be indicated. In most cases, this will be done in relation to the scientific name. There are three primary reasons to attach an annotation to an herbarium specimen in relation to the scientific name:

1. Correct a previous determination.

2. Indicate the current name in use in the case of synonyms.
3. Provide verification of the original determination.

Number 3 above is typically done by a specialist on the specific group of plants. However, this can be done by anyone who feels confident in the determination and feels that verification is needed, such as with plants that are commonly misidentified. In the case of number 3, the scientific name on the original label and annotation label will be the same.

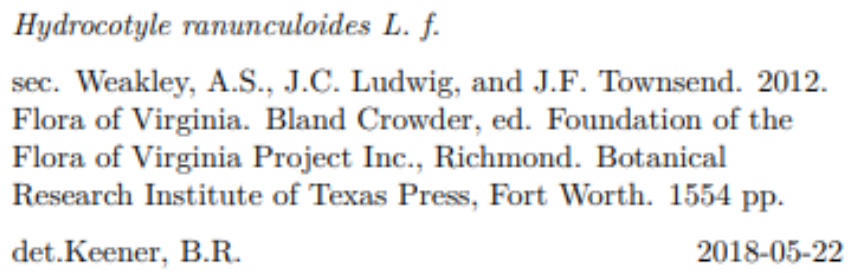
Annotations can also be used to indicate a "mixed sheet" where more than one taxon was inadvertently placed onto a single sheet. An annotation may also document the use of a specimen in a special procedure (e.g., destructive analysis) even when updated taxonomic information is not provided. In that case, a statement such as "leaf material removed for DNA analysis," with an indication of the date and investigator, will appear on the label.

6. **Table 3** indicates the text that should be provided on the label. The terms are based on the Darwin Core metadata standard for biodiversity informatics (version 2011-10-26, http://rs.tdwg.org/dwc/terms/index.htm#theterms).

Table 3. Terms to be included on the annotation label.

Term	Description	Example
scientificName	Genus, species and Authority (genus + specificEpithet + scientificNameAuthorship) or lowest possible rank	*Planta viridis* Barnett
nameAccordingTo	The source used for identification. This can take several different forms such as an author citation, a DOI or the entire bibliographic reference. The abbreviation 'sec.' (from secundum, meaning 'according to') should precede the text. Ideally all of these would be in the NEON standard list of identification sources.	sec. Weber and Wittman 2012
identifiedBy	Who made the determination. The abbreviation 'det.' should proceed this text.	det. M.W. Denslow
dateIdentified	When the determination was made. Best practice is to use the YYYY-MM-DD format.	2013-07-31
identificationRemarks	Any remarks about the determination. This is optional and only used if needed.	Specimen has some characteristics of *Planta alba*.

An example annotation label is shown below (**Figure 14**).



Hydrocotyle ranunculoides L. f.

sec. Weakley, A.S., J.C. Ludwig, and J.F. Townsend. 2012. Flora of Virginia. Bland Crowder, ed. Foundation of the Flora of Virginia Project Inc., Richmond. Botanical Research Institute of Texas Press, Fort Worth. 1554 pp.

det.Keener, B.R. 2018-05-22

Figure 14. Annotation label example.

7. Materials Needed:
    - Archival paper: Any acid free paper can be used, though a high percentage of cotton content is preferred.
    - A computer and laser printer
    - Permanent pen (if being handwritten)
        - Ballpoint and felt tipped pens should not be used
        - Pencil is not recommended since it can be erased
    - Archival glue: A water-soluble pH-neutral adhesive such as white PVA resin
8. Size and Placement

    The original label or any previous annotations should never be altered in any way.

    There is no standard size for annotation labels. They are usually approximately 2.5cm x 10cm. If printing, an easily legible font should be used. A size of 10 points is recommended.

    The annotations should be placed in a blank space near the original label. This is usually to one side or above the label and flush with the right side of the sheet. If other annotations are present, position the new one above the most recent if possible. If it will not fit or the most recent annotation label is in a very unusual position, the annotation label can be placed wherever there is space. A small amount of glue placed on either side of the annotation label should be used. When there is not sufficient blank space, an annotation may be glued only at one end and overlap mounted plant material.

C.6 Mounting

Once specimens are pressed and dry, specimens to be added to the Domain Support Facility herbarium must be mounted. Quality specimen mounting requires skill and patience. The goal is an aesthetically

pleasing and scientifically useful museum piece that will last centuries. All supplies must be of museum quality since the longevity of the specimens is directly related to the substances they contact (**Table 1**). The procedure is outlined below in steps 1 through 8.

1. Evaluate the specimen.
    a. Do not mount a specimen unless it has a complete collection label printed on acid-free paper.
    b. Determine the number of sheets the specimen will need. Most will fit on one sheet. If multiple sheets are needed, make sure each has a copy of the label. Do not discard anything; put extra pieces on a second sheet, in a fragment packet, or set aside as part of a duplicate specimen. Duplicate specimens will be provided with a copy of the collection label and sent, unmounted, to an external archive facility.
2. Arrange the plant, label, and packet on mounting paper before gluing anything down.
    a. Herbarium labels must be flush with mounting paper edges in the bottom right corner (**Figure 15**).
    b. Annotation slips (if present) go directly above or to the left of the label. Leave room around the label at the top and left for future annotation slips.
    c. Archive barcodes should be adhered to the upper left or right corner of the mounting paper (**Figure 16**).
    d. Put a packet on every sheet. Use a packet appropriate to the size of the plant parts that might fall off. Packets can go anywhere on the sheet but must always be at least a quarter of an inch from the edge of the mounting paper.
    e. Some specimens will be tiny because the plants are tiny (e.g. < 3cm). These should not be glued or strapped to the mounting sheet, instead put them in an appropriately-sized fragment packet (**Figure 15**).
    f. Determine which side of the plant to display. Choose the side on which the most features like flowers or fruits are evident and on which more leaves show their upper side (**Figure 17**).
    g. Arrange plant as realistically as possible. Roots or lower part of stem at bottom of sheet, flowers towards the top (**Figure 18**).
    h. Loose pieces should be placed in the packet unless they are very large, in which case they can be mounted next to the rest of the plant. Do not make fake arrangements of parts.
    i. Make sure some leaves are bottom-side up. Turn some over if there are not any already pressed that way. A large single leaf may be cut and mounted with both sides showing or mounted only with strips (**Figure 19** and **Figure 20**).

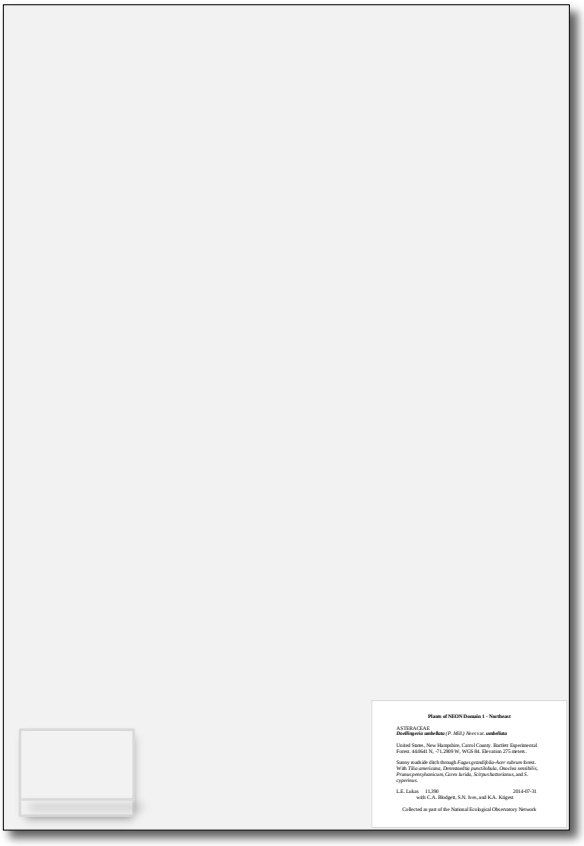


Figure 15. Layout of herbarium sheet before attaching the plant specimen. Collection label is flush with the lower-right-hand corner of sheet. Fragment packet is at least ¼" from edges of the sheet. Leave space either directly above or to the left of the collection label; someday that space will be populated by annotation labels.

Figure 16. Example of placement of the herbarium label (lower right) and archive barcode (upper right).

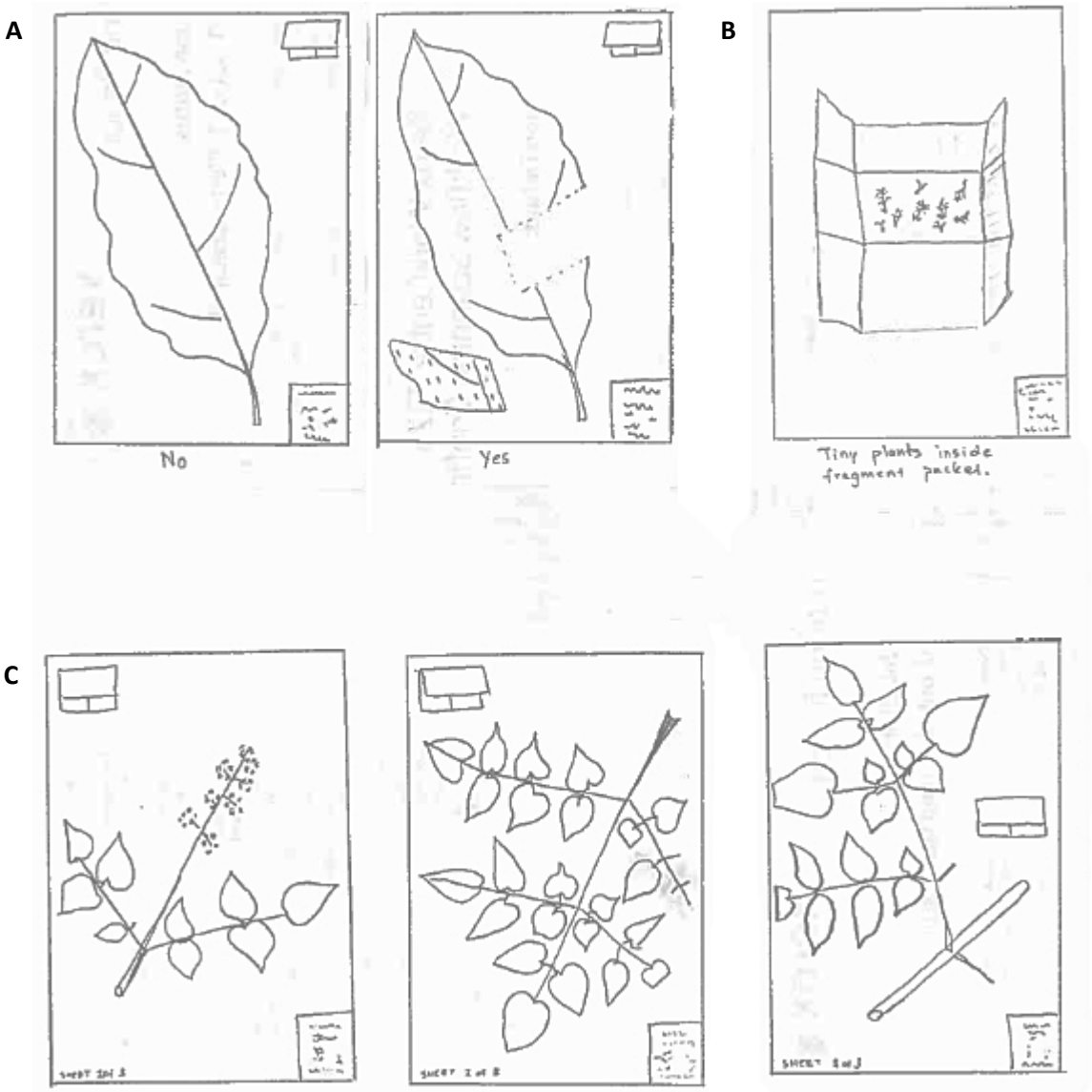


Figure 17. Diagrams illustrating proper and improper arrangement of specimens on mounting paper. Drawing A: A single leaf that was pressed flat takes up one entire page. In order to show both upper- and lower sides of the leaf, cut out a section that includes midrib and margin and attach to page upside-down. Drawing B: The plant specimens are so small that to glue them to the sheet would likely embed them in glue, rendering them too difficult to see clearly; instead, store the individual plants loose inside a fragment packet. Drawing C: To make a specimen from a large plant, it must be split onto three sheets, each sheet numbered with "Sheet 1 of 3" and so on. Make sure to include the top of the plant, section of the middle of the plant, and basal leaves. Additionally, in Drawing C, redundant leaflets have been removed in order to get the plant to fit on the page. Leave a nub at stem attachments so people looking at the specimen will know there was a leaf there.

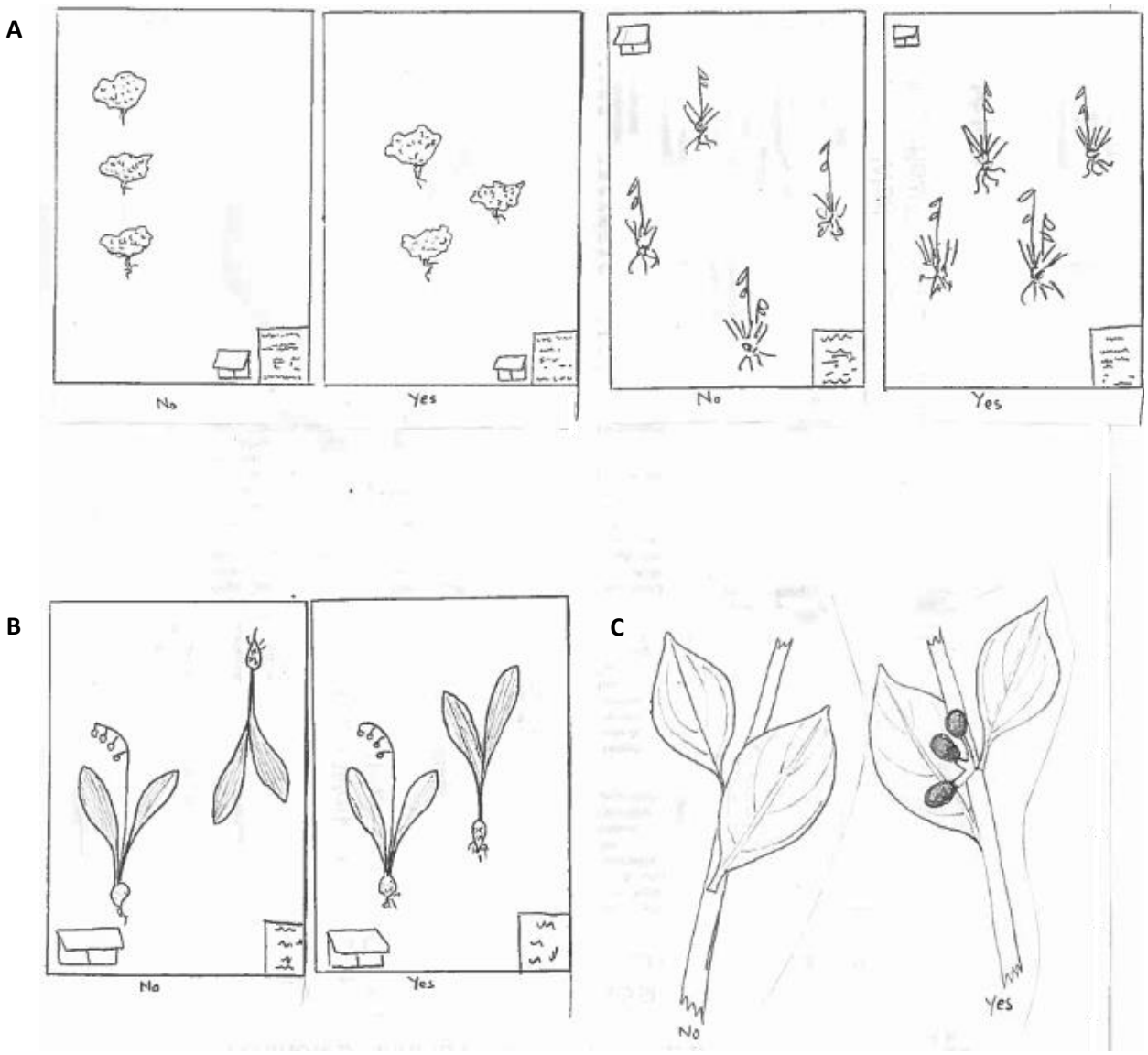
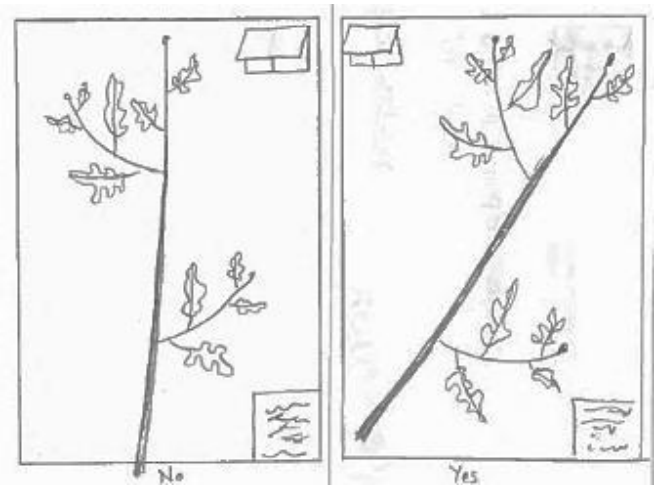
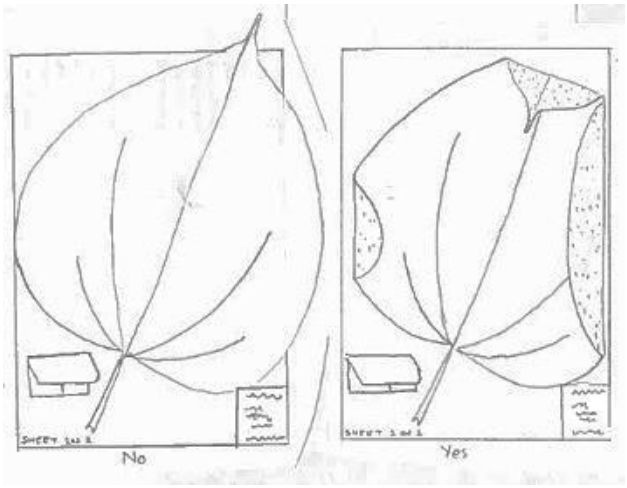


Figure 18. Diagrams illustrating proper and improper arrangement of specimens on mounting paper. Drawing A: When there are multiple small plants to fit on a page, don't arrange them in a contrived manner, rather group them evenly across the page and keep them away from the paper edges. Drawing B: When possible, arrange the plant with roots facing down and the top of the plant toward the top of the sheet. Drawing C: Choose the best side of the specimen to show off; do not allow important details such as fruits and nodes to be obscured by a leaf or other material.

A



B



C

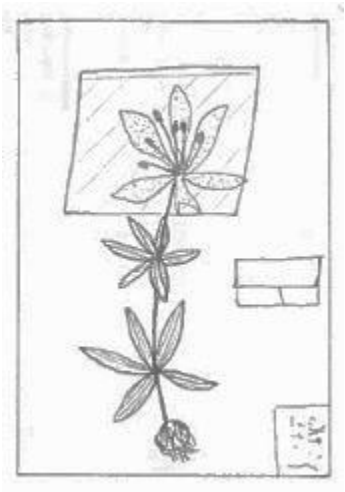


Figure 19. Diagrams illustrating proper and improper arrangement of specimens on mounting paper. Drawing A: Don't let the plant hang over the mounting paper, angle it or cut it to fit instead. Drawing B: To prevent the leaf from hanging over the paper edge and to show underside details, fold leaf. Drawing C: If a specimen only has one flower do not glue it down. Instead, if it is large enough, paint glue on one edge of a sheet of translucent paper, and attach it so it flaps over the flower, keeping the flower protected and in place but still accessible.

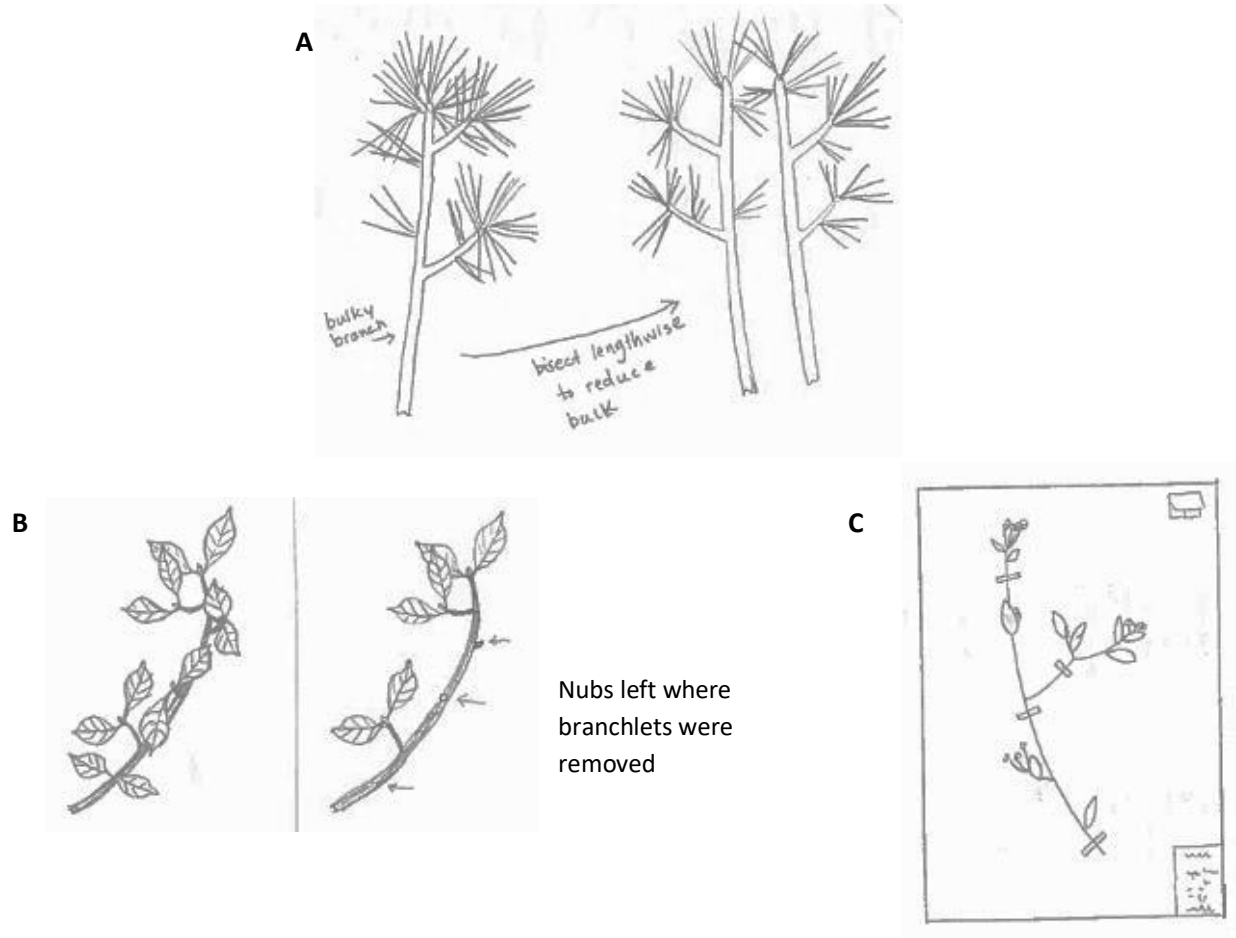


Figure 20. Diagrams illustrating proper and improper arrangement of specimens on mounting paper. Drawing A: Thick, bulky branches may be bisected lengthwise and mounted with cut-side down (or exposed to show color of pith). Drawing B: Sometimes branches are very three-dimensional. One way to help flatten them is to cut off branchlets or leaves that are sticking out at right angles from the mounting paper. Leave evidence of where the branchlets were cut. Drawing C: A specimen with cloth strips. Strips should always be placed near the end of a stiff branch, along long uninterrupted lengths of stem, and any other area where extra stability is needed.

3. Glue the specimen

    a. The glue may be slightly diluted (up to ca. 6 parts glue with 1 part water) if deemed necessary. Don't let the glue sit with the top off of the jar for long periods; it will skim over. Keep the rim of the glue jar clean, and don't shut the lid too tightly. Otherwise it will become glued shut. A plastic weigh boat with glue and a separate water source for as-needed dilution is an option.

    b. When gluing on the label, evenly place a few dots of glue across the back. Do not paint the back of the label with glue, otherwise when it dries the glue will cause the

herbarium sheet to curl up. Position the label flush with the herbarium sheet edges. Cover with a paper towel and smooth it down by rubbing it diagonally.

c. If the plant specimen is especially large, it may be necessary to affix the label only on its right margin and allow it to overlap the specimen. This will allow the plant to be viewed under the label (**Figure 21**).

d. Glue the plant. Try to place glue only on rigid parts of the plant, such as stems and roots. Use dots of glue, and glue sparingly. Avoid gluing down flowers and seeds, as these parts often need to be accessed by botanists. Some herbaria skip the glue all together and only attach plants with cloth strips. This allows future researchers to easily access the specimen's different organs. Metal washers can be placed on plant parts while glue dries.

Figure 21. Diagram of a plant shaped in such a way as to preclude label from being glued down completely. In this case, only the right edge of the label is glued to the mounting sheet, allowing the label to be lifted in order to view all of the plant.

4. Add cloth strips (Figure 22). The purpose of strips is to secure the plant to the mounting paper; over time and with use, the glue on the back of the specimen is likely to fail. Cut strips to appropriate size (thick and long enough to secure the plant but small enough that they obscure as little information as possible), but do not make them too thin. Strips that are extremely thin will not stick to the mounting paper when stressed. Paint the shiny (gummed) side of the strips with a little watered-down glue. Granted, the mounting tape already has a water-activated adhesive on it, but it does not always hold. Affix strips across a few places along the stems (especially at the bottom). Try not to obscure too much of the plant with strips. Affix strips across loose leaves and flowers as necessary.

5. Save all loose pieces and place them in the fragment packet. If there are any loose pressed flowers, put them in a packet. If there are very many of these flowers, a few may be mounted on the sheet.
6. Tips for unusual specimens
    a. Bark: Mount bark so the outer side shows. It is best to affix bark with strips or needle and thread.
    b. Unusually thick stems and other plant parts should go to the right side of the paper, if possible. These parts should be secured with strips or sewed on.
    c. Large, unmountable, plant parts such as seed cones and walnuts should be provided with a copy of the collection label and stored in their own archival resealable plastic baggie or archival box. The corresponding mounted sheet(s) should indicate that parts of the specimen are stored separately (e.g., cone in box). Palms are often very bulky and can be stored in specialized boxes or palm folders. See the Herbarium Handbook for further instructions.
    d. Bryophytes should be slipped into acid-free packets and arranged in bin boxes.
7. Stack the mounted specimens while the glue dries. Stack the specimens in this sequence (from bottom up): old ventilator, mounted plant, wax paper, foam, old ventilator, etc. The wax paper prevents the wet glue from sticking to the foam and cardboard. Put lots of heavy books on top of the finished stack. The glue dries in 8 hours or less (**Figure 23**).
8. Wash all paint brushes in soapy water and lay flat on paper towels to dry. Soap is necessary to get all the glue off.

Figure 22. Diagram depicting the proper use of cloth strips. They are used sparingly and do not cover important diagnostic features.

Figure 23. Newly-glued herbarium specimens stacked to dry. Layers are old ventilators, herbarium specimen on paper, wax paper from the grocery store, and foam bought from a fabric store. The entire stack is weighed down with heavy objects (Ecology issues in this case). Sandbags also work well.

C.7 Additional Mounting Notes

- Over-sized vouchers can be mounted on two herbarium sheets. In this case, two herbarium labels should be created that are labeled (can be added by hand) “1 of 2” and “2 of 2”. A single archive barcode will be produced and go on the first of the two herbarium sheets.

SOP D Storage and Care of Voucher Specimens

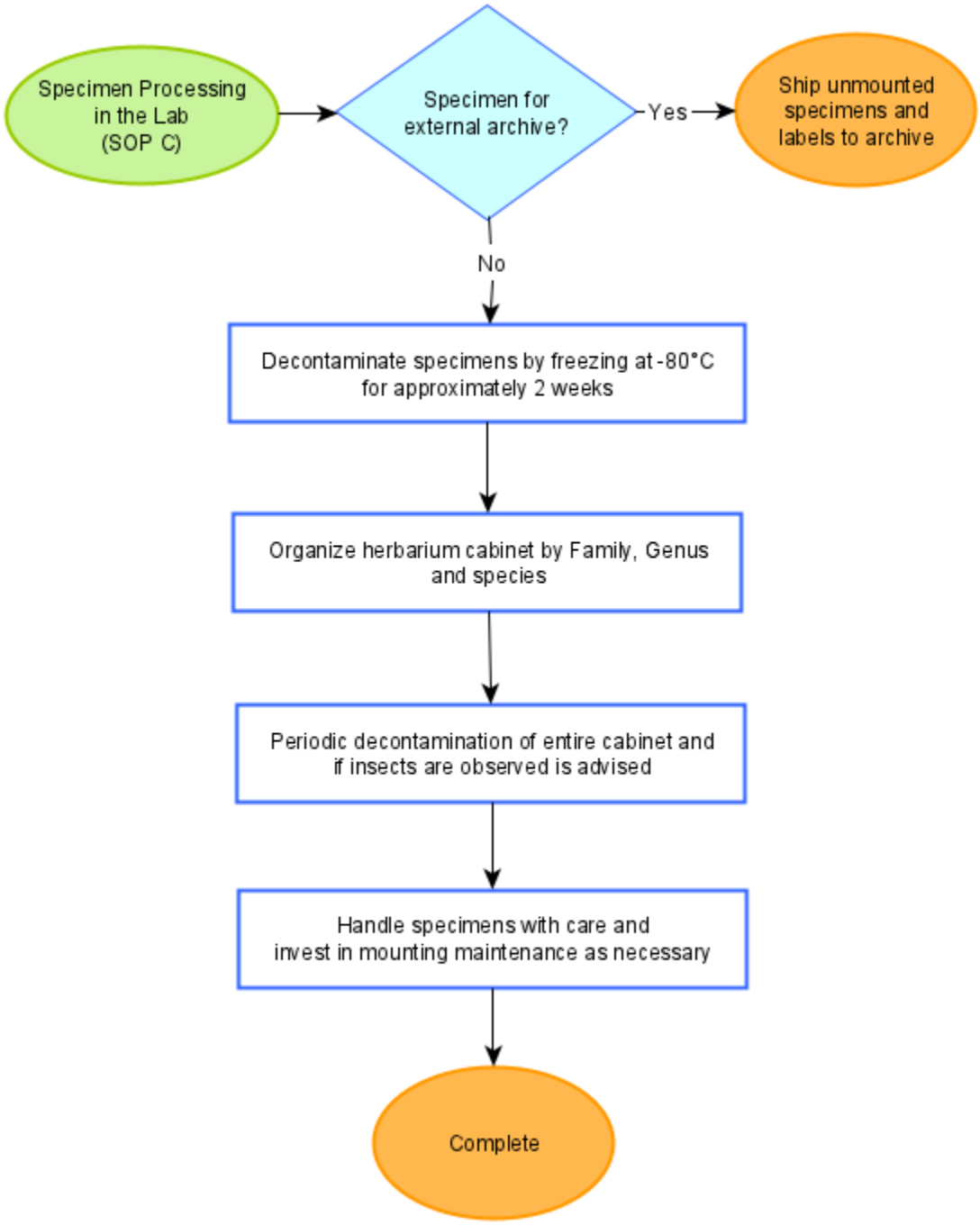


Figure 24. The workflow for storage and care of voucher specimens.

Before moving newly pressed and dried specimens into the herbarium cabinet, they must be sealed in a plastic bag and frozen at -80°C for approximately two weeks, this will kill any insect pests. Climates that are particularly arid may require less time frozen. Consult local experts i.e. herbarium curators who can advise you to an appropriate decontamination schedule for your area. Specimens must cool down rapidly once placed in the freezer so the insects don't have time to adapt, so don't put too big a stack in at once. After sufficient time, remove garbage bags from freezer and allow them to come to room temperature before opening them. Once the bags and their contents are warmed up, the bags may be opened and their contents immediately filed into the herbarium cabinet.

D.1 Herbarium Organization

Most of a Domain's reference herbarium cabinet will be occupied by mounted voucher specimens. Voucher specimens within the herbarium cabinet should be organized by Family, Genus, and then species. Each family gets its own ventilator with a hang-tag printed with the family name. On top of that ventilator are genus folders belonging to that family (**Figure 25**). Each genus folder contains a floppy folder for each species (**Figure 26**). Family hang-tags are printed in bold Calibri size 72 font, all caps. Genus folder labels are printed in bold, italicized Calibri size 48 font, first letter capitalized. Species folders are labelled by hand in pencil. This makes it easier to correct spelling mistakes. On species folders, do not write out the entire genus name; abbreviate it. For example: the species folder for *Rubus idaeus* should read "*R. idaeus*".

Do not let genus folders get too full because the specimens inside will start to warp and break. Keep the contents down to 3cm thickness. If you have more specimens than that, start an additional genus folder.

One or two shelves in the herbarium cabinet should be reserved for unmounted specimens awaiting mounting. Make sure to provide each shelf of unmounted material with a descriptive hang-tag.

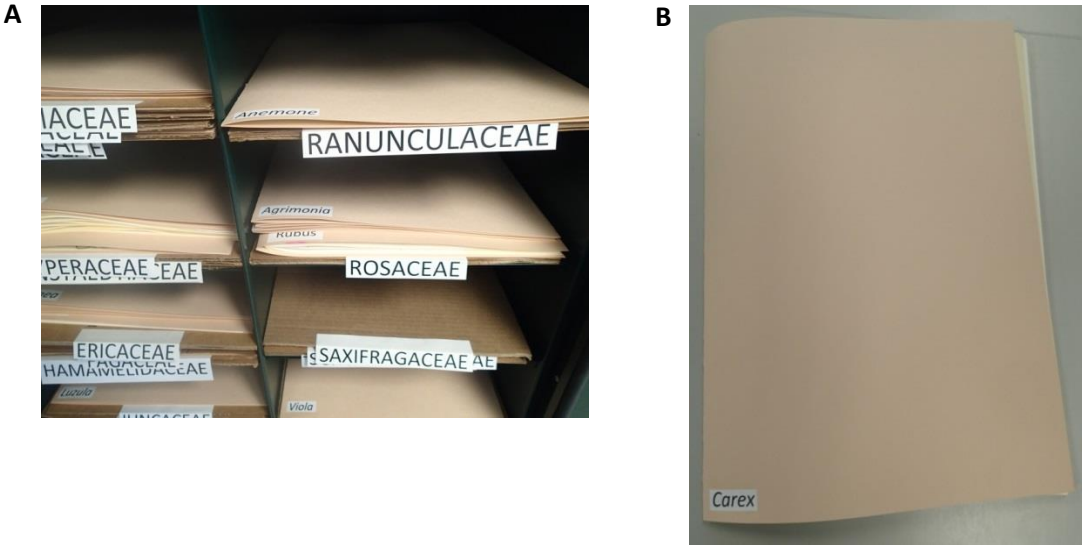


Figure 25. A: Herbarium cabinet with family hang tags taped to ventilators and labeled Genus folders containing species folders. B: Genus folder with Genus name glued to the lower-left corner.

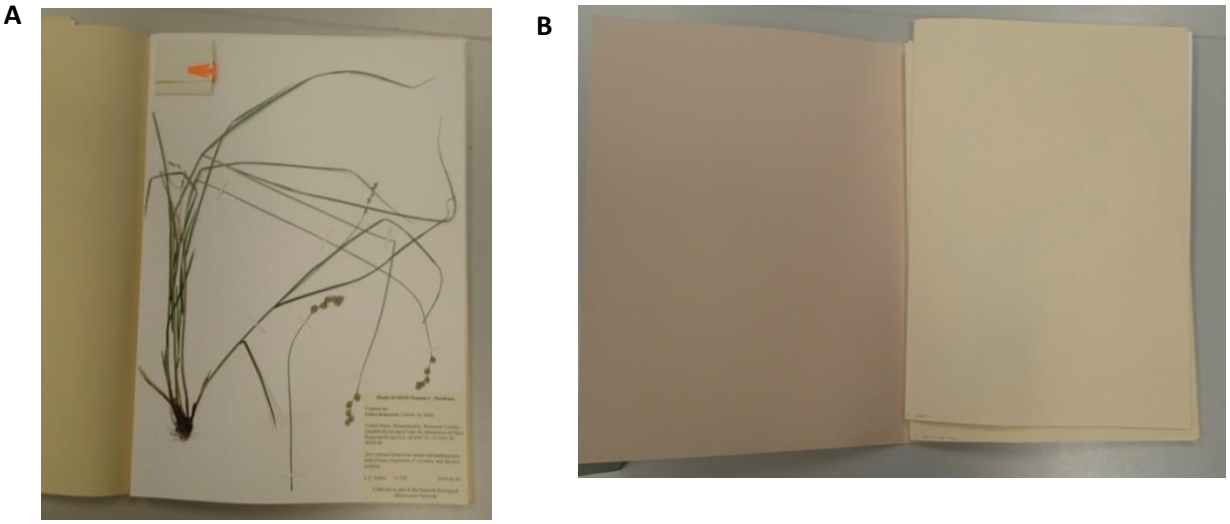


Figure 26. A: Mounted herbarium specimen inside a species folder. B: Species folders inside a genus folder.

D.2 Herbarium Cabinet Maintenance

If possible, the herbarium cabinet should be stored in a room maintained at 40-60% humidity and 20-23°C or lower, environmental conditions inhospitable to insect pests. All contents of the herbarium cabinet should be frozen once every six months, particularly following periods of heavy use. Place all contents in tightly-sealed garbage bags and put in a -80°C freezer for approximately two weeks, this is to kill any insect pests that might otherwise eat herbarium specimens. Climates that are particularly arid may require less time frozen. Consult local experts i.e. herbarium curators who can advise you to an appropriate decontamination schedule for your area. Specimens must cool down rapidly once placed in

the freezer so the insects don't have time to adapt, so don't put too big a stack in at once. After two weeks, remove garbage bags from freezer and allow them to come to room temperature before opening them. Once the bags and their contents are warmed up, the bags may be opened and their contents returned to the herbarium cabinet.

Herbarium pests are usually no longer than two millimeters long, so if you ever see anything crawling around on your specimens, freeze them immediately. While the herbarium cabinet is empty, vacuum it out, focusing especially on crevices and corners. Compressed air may be used if it appears a vacuum isn't removing all debris. If deemed absolutely necessary, treat the empty cabinet with chemical pesticide and tape the door shut for two weeks. Products like Tri-Die dust may be applied along the corners and crevices and then vacuumed up after the end of two weeks. Alternatively, small amounts of PDB can be placed on the top shelves while the cabinet is taped shut for two weeks.

After years of use, herbarium specimens can start to come loose from their paper and will need to be repaired. Proper handling of specimens can prevent some damage, however. Never hold a specimen upside down. Don't flip through a folder of specimens, instead pick specimens up one at a time and avoid bending them. If bits fall off, put them in the fragment packet. When transporting specimens, put them on a rigid surface such as a ventilator.

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APPENDIX A EQUIPMENT

The following equipment is needed to implement the procedures in this document. Equipment lists are organized by task. They do not include standard field and laboratory supplies such as charging stations, first aid kits, drying ovens, ultra-low refrigerators, etc.

Table 4. Equipment list – For pressing, mounting, and labeling herbarium specimens.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
		Bone paper folder	Fold fragment packets	1
Forestry Suppliers; 53875	N	Botany dissection kit	Identify unknown species	1
Forestry Suppliers BioQuip; 53741 3127	N	Cardboard ventilator	Prepare voucher specimen for mounting	Endless
		Clipper (pruning) pole	Collect parts of palms and other trees that are out of human reach	1
Fisher Scientific; 11350121	N	Dissecting microscope	Aid in species identification	1
		Foam sheeting	Dry newly mounted specimens	Variable
Herbarium Supply; GBB1	N	Glue bottle with brush (attached brush optional)	Mount plant voucher specimen	1
		Heavy books or weights	Dry newly mounted specimens	Variable
BioQuip; 1203B3	N	Insect pin #3	Prepare cactus specimen for mounting	Package of 100
		Magic marker	Label collected specimens in the field	A few
		Needle	Sew large fragments to mounting paper	2
		Paint brush – fine tip	Mount plant voucher specimen	3

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
		Paint brush - large	Mount plant voucher specimen	3
Forestry Suppliers Herbarium Supply; 53740 223	N	Paper blotters	Press collected individuals for identification	Variable
BioQuip; 1154F	N	Pen, ethanol-safe	Prepare cactus specimen for mounting	1
Forestry Suppliers BioQuip; 53674 3115	N	Plant press	Press collected individuals for identification	2
		Probe / Dissecting pick	Aid in species identification	2
		Razor blade, flat	Aid in species identification	10
		Scissors or pruning shears	Prepare voucher specimen for mounting	1 ea.
		Small trowel, butter knife, brick hammer, hori hori, or other tool	Dig plants out of ground	1
		Sub-millimeter scale	Aid in species identification	1
		Tweezers, fine-tip	Identify and mount plant voucher specimen	2
		Washer or lead bars	Mount plant voucher specimen	Variable
		Water jar	Mount plant voucher specimen	1
Consumable Items				
		Acid-free printer paper	Label voucher specimens and make fragment packets	1 ream
		Archival plastic bags or boxes (bags are cheaper)	Permanent herbarium specimen storage	Variable

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
		Bin boxes – Herbarium Supply #391 or similar	Permanent bryophyte specimen storage	Variable
		Ethanol, 95%	Press cactus specimens	Variable
		Fragment packets	Store loose pieces of mounted specimen	Endless
		Genus folders	Permanent herbarium specimen storage	Variable
Herbarium Supply; 120	N	Glue, herbarium mounting - Missouri Botanical Garden Type #122	Mount plant voucher specimen	1
Herbarium Supply; 101	N	Herbarium mounting paper	Mount plant voucher specimen	1
Herbarium Supply; 280	N	Herbarium mounting tape	Mount plant voucher specimen	1
Herbarium Supply; 250	N	Newspaper pages	Press collected individuals for identification	Endless
		Palm Folders – Herbarium Supply #137 or similar	Storage of mounted palm specimens	Variable
BioQuip; 1223RB	N	Paper, ethanol-safe label	Prepare cactus specimens for mounting	Package
		Paper towels	Mount plant voucher specimen	Variable
		Plastic or otherwise rust-proof paper clips	Permanent herbarium specimen storage	Variable
		Regular gallon-size reclosable bags or plastic shopping bags	Collect field specimens field	Variable
		Rite-in-the-Rain paper	Field label for collected specimens	Lots
		Species folders	Permanent herbarium specimen storage	Variable
		Thread, unbleached linen (like used for bookbinding)	Mount plant voucher specimen	Lots

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
		Waxed paper	Dry newly mounted specimens	Lots
Resources				
		Field guide, regional flora reference guide and/or key	Identify unknown species	Variable
		NEON label template	Collection label instructions	1
		The Herbarium Handbook	Reference for collecting, pressing, and mounting specimens	1
		Bean 2013 (.pdf from website)	Instructions for palms and bamboo	1

A.1 Suppliers

Herbarium Supply Co. herbariumsupply.com

- Glue – Missouri Botanical Garden Type #122
- Palm folders #137 (optional)
- Polyethylene zipper bags
- Bryophyte packets and cardboards

JoAnn Fabrics or similar

- Foam sheets for drying mounted specimens

University Products universityproducts.com

- Polyethylene zipper bags
- Solid artifact boxes

Blick art supply dickblick.com

- Paper folders

APPENDIX B PRESSING OTHER PLANT TAXA

B.1 Cacti

Cacti should be processed as follows:

1. Slice the cactus lengthwise.
2. If the cactus is thick (e.g., *Echninocereus*), cut the specimen in three or four lengthwise slices (De Groot, 2011).
3. *Opuntia* cladodes should be filleted through the middle. In order to do this, place newspaper around the cladode and then hold down the cladode with cardboard or a piece of plywood while you fillet through the middle (De Groot, 2011).
4. For branching cacti such as *Cylindropuntia*, the ideal method is to slice through the joints lengthwise and preserve the branching pattern (De Groot, 2011).
5. Place an ethanol safe tag on each specimen using a pin with the following information: species, date of collection, site.
6. Completely submerge the specimen in 95% ethanol for 8-24 hours in a sealed container (i.e. cooler). Weigh the specimen down with something heavy to keep it submerged and to pre-flatten it. The amount of time required to preserve in ethanol differs based on size of specimen. It is safe to remove the cacti from the ethanol when the parenchyma tissue has shrunk back from the vasculature. *Feracactus* sp. or *Echinocactus* sp. take around 24 hours. *Opuntia* sp. will take around eight hours (De Groot, 2011).
7. Press each specimen in a plant press as dictated in SOP B, with the following adjustments:
    a. Place the cactus as usual inside a folded newspaper but include a sheet of parchment paper on the cactus' cut side. This will prevent the mucilaginous inside of the plant from sticking.
    b. When you tighten the straps on the press, put some pressure on the specimens such that the spines lay flat, but not too much pressure such that the soft tissue might splay out more than natural (De Groot, 2011).

B.2 Aquatic Plants and Algae

Aquatic plants and algae pose a special challenge as they stick to everything. The best method of pressing these is to:

1. Float the specimen in a pan of clean water.
2. Slip the final mounting paper into the water under the plant.
3. Carefully lift the mounting paper to the surface, capturing the plant in its nice floating arrangement.

4. Place a sheet of waxed paper over the plant, and press the mounting paper, plant, and waxed paper in newspaper with the rest of the plant press.

For a thorough guide to collecting and mounting aquatics see Collecting and Preserving Aquatic Plants by P. Warrington (Warrington, 1994).

B.3 Bamboos and palms

For thorough instructions on how to collect and mount bamboos and palms see the Queensland Herbarium's procedure for processing herbarium specimens (Bean, 2013).

APPENDIX C PRESERVATION OF BRYOPHYTES

Bryophytes (mosses and liverworts) are not pressed because pressing them irrevocably destroys structures necessary to identification. Bryophytes must be dried in paper bags or packets.

Table 5. Equipment and Materials necessary to store bryophyte specimens.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
		Acid-free cartridge paper or similar heavy paper	Bryophyte packets	1 ream
		Acid-free printer paper	Collection label for voucher specimens	1 ream
		Bin boxes – Herbarium Supply #391 or similar	Permanent bryophyte specimen storage	Variable
		Desiccator cabinet	Bryophyte drying	1
		Desiccant packs	Bryophyte drying	50
		Glue, herbarium mounting - Missouri Botanical Garden Type #122	Glue collection label to bryophyte packet	1

C.1 Processing Bryophytes in the Lab

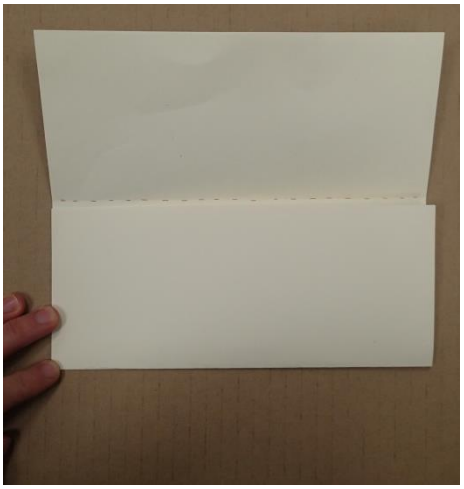
As soon as possible after collecting bryophyte specimens, dry them in the desiccator cabinet. While drying they can be stored either in their craft paper collecting bag or in an archival bryophyte packet. Make sure their collection label data are kept with them. Instructions for folding such packets are below. First, obtain an 8.5 by 11 inch sheet of acid-free cartridge paper:

1

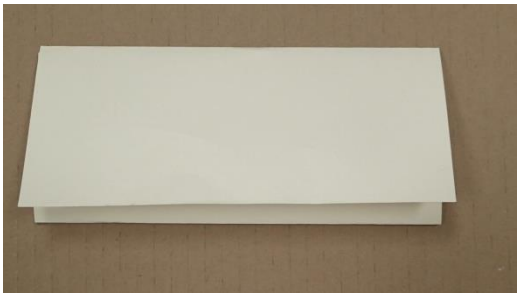
2

1. Start with an 8.5 by 11 inch sheet of cartridge paper.

2. Fold inward along red dotted lines.



3

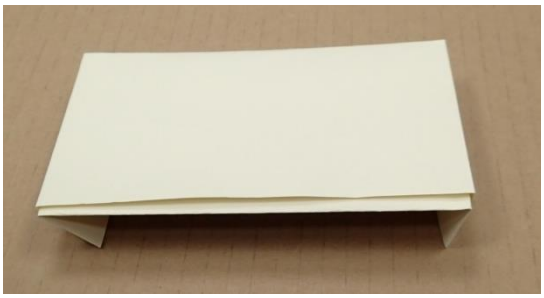


4

3. Folding.

4. Folded into thirds along the dotted red lines.

5



6

1. Fold downward along the dotted blue lines.
2. Folded.

7

8

7. Folded packet with diagram showing where to attach the collection label.

8. Another view of the folded packet.

It may take several days for the moss specimen(s) to fully desiccate. Once dry, place the specimen(s) in a tightly sealed plastic bag and freeze at -80°C for approximately two weeks.

In the meantime, fold archival bryophyte packets and print collection labels, if that hasn't already been done. Glue the printed collection label onto the front flap of the packet.

After two weeks in the freezer is up, remove the bag of specimens from the freezer and allow to come to room temperature unopened. Once the bag and contents have achieved room temperature, place specimens into their bryophyte packets and store them in a bin box filed in the Domain's herbarium cabinet.

As with all herbarium specimens, check the bryophytes for insect damage semiannually and refreeze them annually.

APPENDIX D FOLDING FRAGMENT PACKETS

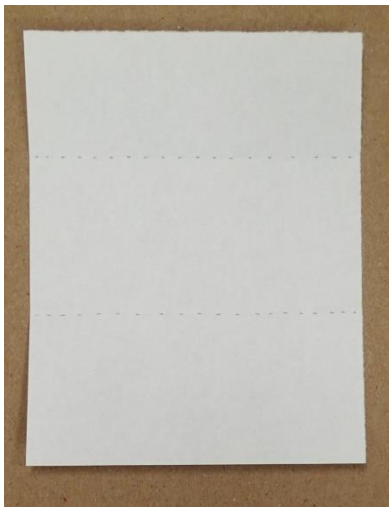
Fragment packets glued to herbarium sheets come in myriad designs. Any packet that can be securely kept shut and that opens completely while glued down is acceptable for inclusion on a mounted herbarium specimen.

Below is a photo tutorial on how to construct a cheap, quick fragment packet.

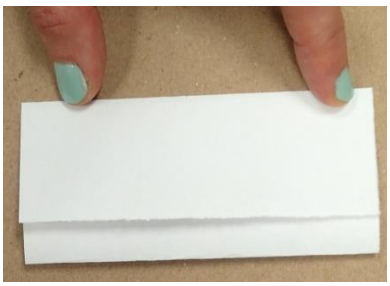
1

2

1. Start with one 8 x 11 inch sheet of acid-free paper.
2. Divide that sheet into four 4 x 5.5 inch pieces.

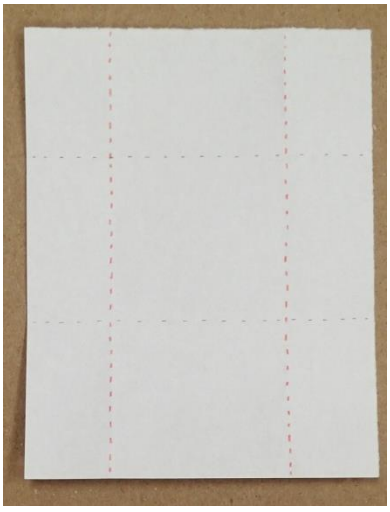


3

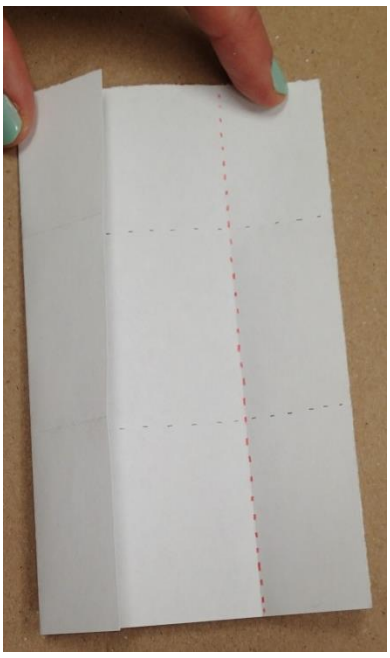


4

3. Fold one of those pieces inward along the gray dotted lines as shown. Don't draw dotted lines on your own paper; it isn't necessary.
4. The folded paper will look like this, with the top flap covering most but not all of the bottom flap.

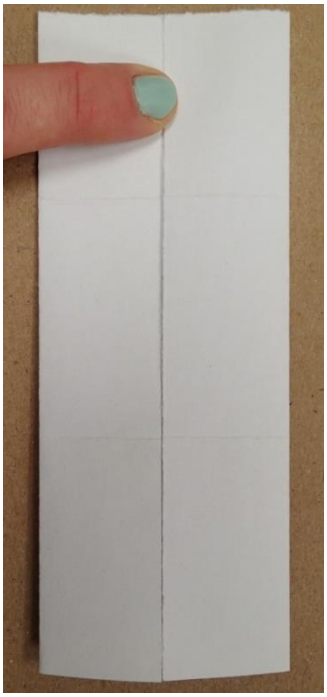


5

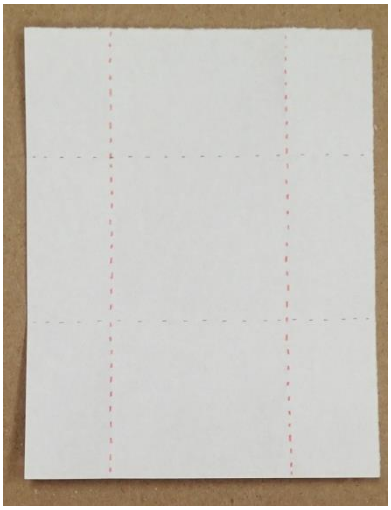


6

5. Open all folds. Now fold inward along the red dotted lines.
6. Folding.

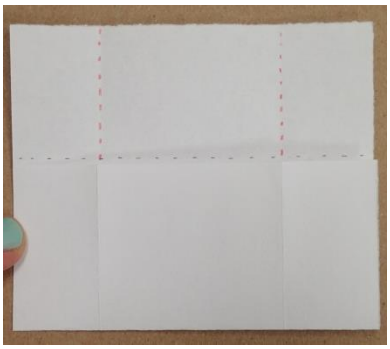


7

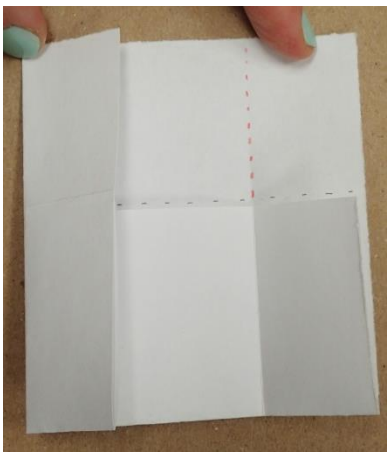


8

7. The paper should now look like this. The folds meet exactly in the middle of the paper.
8. Unfold the paper.

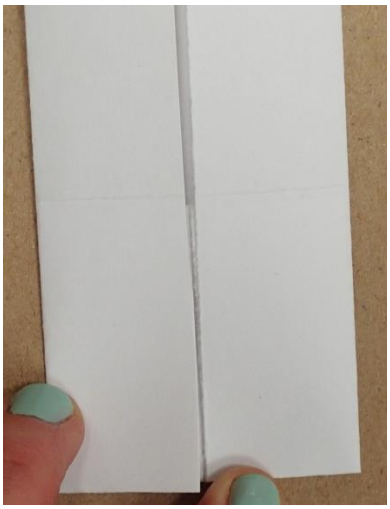


9

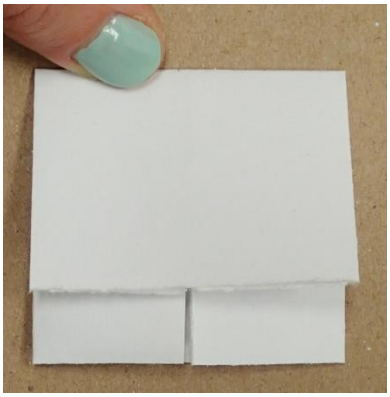


10

9. Fold paper up along the lower gray dotted line.
10. Fold paper inward along the red dotted lines.

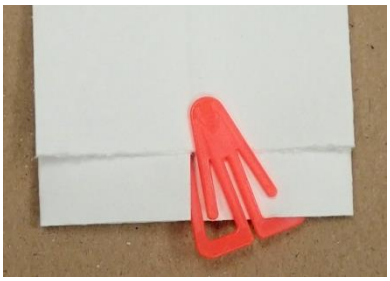


11

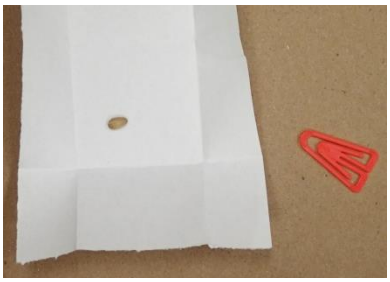


12

13. The paper will now look like this.
14. Fold paper downward along the upper grey dotted line. Now the packet is finished.



13



14

11. After you have glued the packet onto the herbarium sheet, keep it closed with a plastic- or otherwise rust-proof paperclip.
12. This packet has a *Nyssa sylvatica* seed inside!

APPENDIX E COLLECTING TOXICODENDRON SPECIMENS

Table 6. Equipment and materials required for a team of two to minimize exposure to toxic oils from *Toxicodendron* spp. that must be collected.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
Hand clippers	N	Same item type as indicated in equipment lists	Labeled hand clippers, dedicated to Toxicodendron clipping	
Cotton gloves	N	http://www.globalindustrial.com/p/safety/hands/cottoncanvasgloves/anchor-4501v-8-oz-cotton-canvas-knit-wrist-1110	Cotton gloves, single use	Box of 12
Disposable PPE outer-wear	N	Coveralls; http://disposablegarments.com/shop/kool guard/koolguard-coveralls/	Disposable PPE outer-wear	Case of 24
Large, single-useplastic bags	N	Trash bag or large Ziploc type bag	Large, single-use plastic bags	
Cleanser, urushiol specific	N	Tecnu or equivalent	Cleanser, urushiolspecific	1

E.1 Minimizing Exposure to Toxic Oil in the Field and Lab

The following are best-practice techniques for minimizing exposure to toxic oil during collection of *Toxicodendron* species.

1. Prior to field work:
    a. Gather equipment in Table 2.

2. To handle Toxicodendron in the field:
    a. Wear cotton gloves and dispose after single use. Toxic oils can pass through nitrile or latex gloves.
    b. Use a pair of clippers dedicated solely to clipping *Toxicodendron* spp, and clean with Tecnu (or equivalent) after each use. Store separately from other clippers to prevent accidental contact.
    c. Store the collected *Toxicodendron* specimen in its own plastic bag, so it does not contaminate other plants collected that day.
    d. Store and transport contaminated gloves, PPE, and clippers in their own large plastic bag.
    e. Wear a thin outer layer of disposable PPE over clothes and shoes.

3. After field work is complete, wash clothing according to these guidelines or similar:
    a. While handling and loading unwashed clothing exposed to toxic oils, wear gloves or use a clean cloth to prevent direct contact between your skin and the clothing.
    b. Wash with ordinary laundry detergent at the highest recommended water temperature.
    c. Do not overload the machine; the clothes must be allowed to agitate freely.

4. To process Toxicodendron biomass in the laboratory:
    a. Wear a clean pair of cotton gloves when pressing the specimen, and clearly label its pressing paper with a warning. If the *Toxicodendron* specimen gets mounted, throw away the pressing paper, do not re-use it.
    b. Minimize potential spread of toxic oil by putting pressed *Toxicodendron* specimens into the same drying oven every time.
    c. When drying is complete, clean drying oven shelves used for drying *Toxicodendron* biomass bags with hot water and Tecnu. Wear appropriate PPE when cleaning.